THE 11TH BENEFICIAL MICROBES CONFERENCE

PRE- AND PROBIOTICS FOR LIFELONG Human AND Animal HEALTH

ABSTRACTS OF LECTURES & POSTERS

3-5 NOVEMBER 2025

AMSTERDAM
THE NETHERLANDS

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Key to the abstracts of lectures and posters:

- abstracts of lectures and posters are grouped separately
- lectures are grouped according to the daily programme
- posters are grouped in an alphabetical order according to the presenting/corresponding author

BENEFICIAL MICROBES CONFERENCE SERIES

The Beneficial Microbes Conference series started in 2008. During the years, it has become an important meeting point for academia and industry providing a reference source for professionals who want to be updated on the advances in research on beneficial microbes (probiotics, prebiotics, postbiotics, synbiotics, fermented foods, …) for human and animal health across the lifespan. The conference promotes the creation of new initiatives for the customised application of beneficial microbes in food, feed, and healthcare in a networking environment.

THE PROGRAMME

- The 11th Beneficial Microbes Conference provides an overview of the latest scientific results and future developments related to beneficial microbes and their importance to human and animal health. Topics covered include: the role of AI in beneficial microbes research; research trends in next generation probiotics; prevention and treatment of multiple diseases; beneficial microbes for human and animal health; fermented foods and health; beneficial microbes and brain function; and more.

- The International Probiotics Association (IPA) will provide a high-level overview of current initiatives and outline potential future directions for the biotics field.

- The silver sponsor Bialtec presents a dedicated lunch break session. You are invited to join and discover how microbiome engineering is reshaping animal nutrition toward more efficient, sustainable, and antibiotic-free systems.

- The science battle is a valued part of the conference. This year’s science battle is about “Genetically modified probiotics – To ban or not to ban?”. Two speakers will take a different side of the topic followed by a panel discussion and Q&A with the audience.

CONFERENCE CHAIRS

Dr Koen Venema	Beneficial Microbes Consultancy, the Netherlands
Dr Guus Roeselers (co-chair)	Unilever Foods, the Netherlands

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George Paraskevakos	International Probiotics Association (IPA), USA
Dr Henrik Munch Roager	Department of Nutrition, Exercise and Sports, University of Copenhagen, Denmark
Dr Robert Steinert	dsm-firmenich, Switzerland and University of Adelaide, Australia

PROGRAMME AT A GLANCE

11TH BENEFICIAL MICROBES CONFERENCE

Monday 3 November 2025

12:45 - 13:00	Opening of the **11th Beneficial Microbes Conference**
13:00 - 14:00	SESSION 1 *The role of AI in beneficial microbes research*
14:00 – 16:05	SESSION 2 *Next generation probiotics: Research trends*
16:05 – 16:30	Networking break & Poster viewing
16:30 – 18:00	SESSION 3 *IPA Expanding Global Leadership in the Biotics Space*
18:00 – 19:00	Happy hour
19:00	End of day 1

Tuesday 4 November 2025

08:30 – 10:35	SESSION 4 *Beneficial microbes: Prevention and treatment of multiple diseases*
10:35 – 11:00	Networking break & Poster viewing
11:00 – 12:45	SESSION 5 *The focus on pre- and postbiotics*
12:45 – 14:00	Lunch break
13:00 – 14:00	LUNCH BREAK SESSION *Next-generation probiotics: Precision tools for microbiome engineering*
14:00 – 15:45	SESSION 6 *Beneficial microbes for human and animal health*
15:45 – 16:15	Networking break & Poster viewing
16:15 – 17:35	SESSION 7 *Fermented foods and health*
17:35	End of day 2

Wednesday 5 November 2025

09:00 – 10:30	SESSION 8 *The battle: Genetically modified probiotics – To ban or not to ban?*
10:30 – 11:00	Networking break
11:00 – 12:45	SESSION 9 *Brain health and beneficial microbes: What's up, doc?*
12:45 – 13:00	Lessons learned
13:00	Closing of the **11th Beneficial Microbes Conference**

MONDAY 3 NOVEMBER 2025

CONFERENCE PROGRAMME

12:45 **Introduction to the 11th Beneficial Microbes Conference**
Dr Koen Venema, *conference chair*

SESSION 1
The role of AI in Beneficial Microbes Research

Chair: Dr Koen Venema, *Beneficial Microbes Consultancy, the Netherlands*

13:00 **AI-driven multi-omics analysis of the microbiome: From raw data to clinical insight**
Dr Laura Judith Marcos Zambrano, *IMDEA Nutrition Institute, Spain*

13:20 **Applications of machine learning for the functional analysis of (meta-)genomic data of gut microbes**
Dr Georg Zeller, *Leiden University Center for Infectious Diseases, LUMC, the Netherlands*

13:40 **The role of nutrition in IBD managemen: From beneficial microbes to AI approaches**
Prof. Amedeo Amedei, *Department of Experimental and Clinical Medicine, University of Florence, Italy*

SESSION 2
Next Generation Probiotics: Research Trends

Chair: Dr Koen Venema, *Beneficial Microbes Consultancy, the Netherlands*

14:00 **The science behind *Akkermansia muciniphila* and its future perspective as beneficial microbe**
Prof. Clara Belzer, *Laboratory of Microbiology, Wageningen University & Research, the Netherlands*

14:20 ***Akkermansia muciniphila* in infectious disease: A new target for this next-generation probiotic?**
Dr Cormac Gahan, *School of Microbiology, University College Cork, Ireland*

14:40 **One species, many strains: how capturing intra-species diversity may shape probiotic efficacy – the case of *Lactobacillus crispatus***
Prof. Remco Kort, *Amsterdam Institute for Life and Environment, VU Amsterdam, the Netherlands*

15:00 **Linking intestinal permeability and the gut microbiome in cirrhosis: The role of *Phascolarctobacterium* as a potential next generation probiotic**
Rosa Haller, *Department of Internal Medicine, Medical University of Graz, Graz, Austria*

15:20 **Mining the gut microbiota for next-generation probiotic candidates in metabolic diseases and beyond**
Dr Valentina Tremaroli, *Department of Molecular and Clinical Medicine, University of Gothenburg, Sweden*

15:40 **Translating adult microbiome complexity into health-focused biotics innovation**
Dr Sudarshan Shetty, *Danone Global Research & Innovation, the Netherlands*

16:00 **Poster pitch: *Rothia* spp. as potential next-generation oral probiotics: Investigation of nitrate reduction and antimicrobial properties**
Dr Isabela M. Fernandes de Oliveira, *Department of Microbiology and Systems Biology, TNO, the Netherlands*

16:05 **Networking break & Poster viewing**

SESSION 3

IPA Expanding Global Leadership in the Biotics Space

In 2025, the International Probiotics Association (IPA) sharpened its role in uniting the global biotics community, advancing regulatory dialogue, and reinforcing the scientific credibility of pre-, pro-, and postbiotics. Amid rapid innovation and evolving policy, IPA continues to serve as both a convener and a guardian of standards. The session will provide a high-level overview of IPA's current initiatives and outline potential future directions for the biotics field.

Chair: George Paraskevakos, *International Probiotics Association (IPA), USA*

16:30 **Introduction**
George Paraskevakos, *IPA, USA*

16:40 **Probiotic in Europe: Navigating a complex and evolving landscape**
Rosanna Pecere, *Executive Director at IPA Europe, Belgium*

17:00 **Commercial prebiotic manufacturing: Implications for health, product efficacy, and regulatory approvals**
Sandra Saville, *Director of Education and Communication at IPA, Canada*

17:20 **Eating the elephant: IPA's first bite in shaping a credible future for DTC stool microbiome testing**
Dr Jessica Younes, *Scientific Director at IPA, Canada*

17:40 **Novel bacteria-derived compounds to tackle neurodegeneration: Lessons form the nematode *Caenorhabditis elegans***
Dr Konstantinos Palikaras, *Medical School, National and Kapodistrian University of Athens, Greece*

18:00 **Happy hour, with drinks & snacks**

19:00 **End of day 1**

TUESDAY 4 NOVEMBER 2025

SESSION 4

Beneficial Microbes: Prevention and Treatment of Multiple Diseases

Chair: Dr Henrik Munch Roager, University of Copenhagen, Denmark

08:30 **Antidiabetic agents as gut microbiome modulators for disease suppression**
Prof. Yun-Gi Kim, *Department of Microbiology, Kitasato University, Japan*

08:50 **Human milk oligosaccharide metabolism by *Clostridium* species suppresses inflammation and pathogen growth**
Dr Jon Chapman, *Translational and Clinical Research Institute, Newcastle University, UK*

09:10 **Probiotic intervention in age-related muscle decline: A clinical perspective**
Sylvie Binda, *Lallemand Health Solutions, France*

09:30 **Precision microbiome therapeutics for intractable disorders**
Prof. Sin-Hyeog Im, *Department of Life Sciences, Pohang University for Science and Technology, Korea*

09:50 **Integrated multi-omics of the gut microbiome in Parkinson's disease reveals a functional disruption of bacterial motility and cross-feeding**
Dr Rémy Villette, *Luxembourg Centre for Systems Biology, University of Luxembourg, Luxembourg*

10:10 **Data from within: Navigating the small intestine with HintSight**
Dr Thomas Soranzo, *Pelican Health, France*

10:30 **Poster pitch: How to study the effects of dietary lipids on the small intestinal microbiome? The TAPIR proof-of-concept study**
Dr Lonneke Janssen Duijghuijsen, *Wageningen Food & Biobased Research, Wageningen University & Research, the Netherlands*

10:35 **Networking break & Poster viewing**

SESSION 5
Beneficial Microbes: The Focus on Pre- and Postbiotics

Chair: Dr Kaouther Ben Amor, *Danone Global Research & Innovation Center, the Netherlands*

11:00 **Targeted microbiome manipulation: From metabolic models to precision prebiotics**
Dr Georgios Marinos, *Institute of Experimental Medicine and CAU Innovation GmbH, Kiel University*

11:20 **Structure matters: How different fibres lead to differential modulation of the gut microbiota**
Dr Koen Venema, *Wageningen Food and Biobased Research, Wageningen University & Research, the Netherlands*

11:40 **Postimmunobiotics: promising alternatives to combat viral infectious diseases in livestock**
Prof. Haruki Kitazawa, *Graduate School of Agricultural Science, Tohoku University, Japan*

12:00 **Restoring balance: pro- and postbiotic strategies to counter antibiotic-induced dysbiosis in a canine gut model**
Dr Charlotte Deschamps, *UMR 454 MEDIS, University Clermont Auvergne – INRAE, France*

12:15 **Polyphenols: A new generation of prebiotics?**
Dr Linda Lopez, *Givaudan, Switzerland*

12:30 **Human clinical results on immunity using a postbiotic gummy supplement – A case study for the use of inanimate microbes in a food application**
Dr Aurélie Goux, *Cargill, France*

12:45 **Lunch break**

LUNCH BREAK SESSION
Next-generation probiotics: Precision tools for microbiome engineering

Presented by Bialtec

13:00 - 14:00
Discover how microbiome engineering is reshaping animal nutrition toward more efficient, sustainable, and antibiotic-free systems. In this lunch break session, Bialtec experts will share cutting-edge research on replacing antibiotic growth promoters with microencapsulated probiotics, modulating the intestinal resistome, and developing synthetic microbiomes as a new generation of functional feed additives.

Combining biotechnology, anaerobic fermentation, and gastrointestinal simulators, this session unveils practical and scientific strategies to redesign gut health from a microbiological and sustainability-driven perspective.

TUESDAY 4 NOVEMBER 2025

SESSION 6
Beneficial Microbes for Human and Animal Health

Chair: Dr Frédérique Chaucheyras-Durand, *Lallemand SAS and Clermont Auvergne University, France*

14:00 **Unveiling the probiotic bacteria of the honeybee**
Dr. Jorge Gutierrez-Merino, *Faculty of Health and Medical Sciences, University of Surrey, UK*

14:15 **Phage therapy in poultry farming: Natural solutions against antibiotic resistance**
Dr Sandra Sevilla-Navarro, *CECAV, Spain*

14:35 **Systematic profiling of the composition and function of the chicken gut microbiome under distinct microbial based solutions**
Dr Maxine Ty, *University of Toronto and The Hospital for Sick Children, Canada*

14:55 **Microencapsulated probiotics for piglets: Precision microbiome engineering and real-world resilience**
Dr Juan Camilo Arroyave, *Bialtec, Colombia*

15:10 **The rise of probiotic yeasts**
Dr Kimmo Makinen, *Gnosis by Lesaffre, France*

15:25 **Unlocking the microbiome signature of endometriosis: From dysbiosis and metallomics to targeted interventions**
Karen Pendergrass, *Microbiome Signatures, Cyprus*

15:40 **Poster pitch: Flow cytometry: A crucial tool for assessing and visualizing yeast-bacteria adhesion**
Dr Marion Schiavone, *Lallemand and Toulouse Biotechnology Institute, France*

15:45 **Networking break & Poster viewing**

SESSION 7
Fermented Foods and Health

Chair: Dr Guus Roeselers, *Unilever Foods, the Netherlands*

16:15 **Neuromodulatory potential of fermented foods**
Ramya Balasubramanian, *APC Microbiome Ireland, University College Cork, Ireland*

16:35 **Distinct modulatory effects of high-fibre and fermented-food diets on gut microbiota, immune function, transit time, and sleep quality in a citizen science randomized controlled trial**
Maartje van den Belt, *Wageningen Food and Biobased Research, Wageningen University & Research, the Netherlands*

16:55 **A long history of dairy: Gut microbiota and traditional dairy consumption in Mongolia**
Dr Akari Shinoda, Microbiome Science Department, *Max Planck Institute for Evolutionary Anthropology, Germany*

17:15 **Behaviour of the microbiome found in raw milk goat cheese during transit through the gastrointestinal tract as simulated in the TIM-1 system**
Doetie Trinks, *Doetie's Geiten, the Netherlands*, and Dr Maitrayee Chatterjee, *InnoGI Technologies, the Netherlands*

17:35 **End of day 2**

SESSION 8

The Battle: Genetically Modified Probiotics – To Ban or Not to Ban?

How to look at genetically modified probiotics from different perspectives? Two speakers will take a different side of the topic followed by a panel discussion and Q&A with the audience.

Chair: Dr Koen Venema, *Beneficial Microbes Consultancy, the Netherlands*

09:00 **Chair's introduction to the debate**

09:05 **The next generation of probiotics are GMM**
Dr Hugo Roume, *Lesaffre International, France*

09:25 **We need good governance as a precondition to GM probiotics: Lessons from the GM plants controversy**
Prof. Philip Macnaghten, *Knowledge, Technology and Innovation Group, Wageningen University & Research, the Netherlands*

09:45 **Debate and Q&A with the audience**

10:30 **Networking break**

SESSION 9

Brain health and Beneficial Microbes: What's Up, Doc?

Chair: Prof. Michiel Kleerebezem, *Wageningen University & Research, the Netherlands*

11:00 **Developing neural networks to study aging and associated diseases through changes in the microbiome**
Dr Adrían Martín-Segura, *IMDEA Nutrition Institute, Spain*

11:20 **High-throughput screening of fermented foods for health benefits: A scalable *in vitro* approach**
Dr Eric Hester, *NIZO food research, the Netherlands*

11:40 **Initial insights into the microbiota-gut-brain axis in extremely preterm infants**
Dr Hendrik Niemarkt, *Neonatal Intensive Care Unit, Máxima Medical Centre, the Netherlands*

12:00 **Prebiotics as promoters of cognitive resilience: A new perspective**
Dr Franco Ruiz, *Translational Research Center in Gastrointestinal Disorders, KU Leuven, Belgium*

12:20 **Precision synbiotics targeting the gut-brain axis in autism spectrum disorder and chronic fatigue**
Dr Robert Steinert, *Health, Nutrition & Care, dsm-firmenich, Switzerland*

12:45 **Lessons learned**
Dr Koen Venema, *conference chair*

13:00 **Closing of the 11th Beneficial Microbes Conference**

Take your packed lunch to eat along the way!

LECTURE ABSTRACTS

SESSION 1
THE ROLE OF AI IN BENEFICIAL MICROBES RESEARCH

AI-DRIVEN MULTI-OMICS ANALYSIS OF THE MICROBIOME: FROM RAW DATA TO CLINICAL INSIGHT

Laura Judith Marcos Zambrano

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Microbiome research has been mostly based on single-omics methods, such as metagenomics for many years. This methodology provides valuable insights into the genetic potential of microbial communities and has been crucial for describing community composition and functional capacity. However, it only offers a static picture and cannot fully capture the dynamic and functional interactions within microbial ecosystems, or between microbes and their host. Multi-omics approaches which combine different layers of biological information, such as genomics, transcriptomics, proteomics, and metabolomics, grants a more complete and system-level understanding of microbial communities. These multi-omics integration allows to link genetic potential to functional outputs, making it easier to study how microbes interact with each other and with their host. Building co-occurrence networks from these datasets also helps to uncover causal relationships across molecular layers. Taken together these approaches provide a deeper view of the mechanisms that connect microbial activity to host physiology, something that single-omics methods alone cannot achieve.

On the other hand, advances in artificial intelligence (AI) and machine learning (ML) have provided new tools to analyse the complexity of microbiome data. Multi-omics datasets are large, diverse, and noisy, making it challenging to interpret with traditional methods. AI and ML algorithms are well suited for these tasks because they can recognize patterns, identify key features, and build predictive models. Particularly, in microbiome research, AI is used from assigning taxonomy and detect functional genes to more complex analysis such predict microbial interactions, and modelling host-microbiome dynamics. We applied these approaches to study the role of the gut microbiota in non-responsive celiac disease (NRCD), a condition where patients continue to experience symptoms despite following a strict gluten-free diet. Using microbial co-occurrence networks combined with metabolomic data in a ML framework, we investigated how microbiome structure and function relate to disease persistence. Our results showed that symptoms were associated with changes in biomarkers of mucosal integrity, as well as with distinct gut microbiome profiles in NRCD patients compared to controls.

The case of NRCD demonstrates how these methods can shed light on disease mechanisms and point toward new strategies for diagnosis, monitoring, and treatment. Looking ahead, we aim to apply these integrative approaches within the GutPlantHealth project to evaluate how sustainable plant-based diets affect both human health and environmental impact. By linking dietary patterns, microbiome function, and ecological outcomes, this work seeks to provide evidence-based strategies that support healthier people and a healthier planet.

APPLICATIONS OF MACHINE LEARNING FOR THE FUNCTIONAL ANALYSIS OF (META-) GENOMIC DATA OF GUT MICROBES

Georg Zeller

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How our gut microbiota breaks down dietary and host-derived (macro-)molecules has far-reaching consequences, both for the microbes and the host. Microbial carbohydrate-active enzymes (CAZymes) are a key class of enzymes, acting not only on dietary fibre, but also on host-derived molecules, e.g., from intestinal mucus. CAZymes play critical roles in mediating the beneficial effects of fibre; conversely gut microbial degradation of mucus in the absence of sufficient fibre intake is thought to promote gastrointestinal diseases, such as colon cancer and inflammatory bowel disease. To be able to routinely identify and quantify CAZymes in metagenomic data, we have developed the software tool Cayman (https://github.com/zellerlab/cayman) and have curated a hierarchical substrate annotation scheme that facilitates the interpretation of metagenomic CAZyme profiles, for instance to compare mucin and fibre utilization capacity of the microbiota.

When we applied Cayman to a meta-analysis comparing the metagenomes of colorectal cancer (CRC) patients to controls, we identified a consistent pattern of CAZyme enrichment and depletion in CRC patients across studies from three different continents. CRC was associated with a significant depletion of fibre-targeting CAZymes, while GAG- and mucin-targeting CAZymes were significantly enriched. These results are in line with dietary epidemiological risk factors for CRC development, such as insufficient fibre intake and excessive red meat consumption. To identify a taxonomic microbiome signature of CRC in the same meta-analysis setting, we utilized the machine-learning toolbox SIAMCAT developed in our lab. In this signature, several proteolytic genera, such as *Fusobacterium*, *Parvimonas*, *Porphyromonas* and *Peptostreptococcus*, were enriched in CRC. In contrast to these, the fibre-degrading genus *Lachnospira* was depleted in CRC metagenomes. We extensively validated the machine-learning classifier underlying this signature and demonstrated it to be highly robust to technical and geographic variability in microbiome data collected globally from 27 studies. We finally explored how this CRC microbiome signature is associated with dietary habits and detected an anticorrelation with fibre intake. What’s more, a re-analysis of fibre intervention studies revealed that the microbial CRC signature can be alleviated, primarily through promoting the abundance of CRC-antagonizing *Lachnospira*.

Taken together our results demonstrate how CAZyme analysis can reveal functional microbiome plasticity associated with either detrimental or beneficial effects of gut microbes, in turn promoting or supressing disease.

THE ROLE OF NUTRITION IN IBD MANAGEMENT: FROM BENEFICIAL MICROBES TO AI APPROACHES

Amedeo Amedei

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Inflammatory bowel diseases (IBD), including ulcerative colitis and Crohn’s disease, are chronic, relapsing disorders with rising global prevalence and variable incidence. Their pathogenesis involves complex interactions among genetic predisposition, host immunity, and environmental factors, leading to dysfunctional immune responses and persistent intestinal inflammation. In addition to elevated cytokine production and altered microRNA expression, accumulating evidence document alterations in both faecal and ileal microbiome of IBD patients. This imbalance is characterized by an overrepresentation of pro-inflammatory and mucin-degrading bacteria (e.g., *Fusobacterium* spp., *Escherichia* spp.) alongside a decrease in short-chain fatty acid (SCFA)-producing bacteria (e.g., *Roseburia* spp., *Faecalibacterium* spp.), which are critical for maintaining epithelial integrity and intestinal homeostasis

Diet has emerged as a pivotal environmental determinant in IBD onset and progression. Research has advanced from broadly dietary guidelines toward mechanistic insights into how nutrients, dietary patterns, and microbial metabolites modulate mucosal immunity and inflammatory responses. Beneficial microbes and their metabolic products, mainly SCFAs, are increasingly recognized as central mediators of gut health, supporting novel preventive and therapeutic strategies through dietary modulation and the use of prebiotics, probiotics, and postbiotics. The diet–microbiota axis is inherently bidirectional: while specific nutrients can promote microbial balance and intestinal resilience, others exacerbate dysbiosis; conversely, microbial metabolism generates bioactive compounds that directly influence epithelial integrity and immune regulation.

Despite these advances, translating this complexity into personalized nutritional strategies remains a major challenge. Patient heterogeneity, the scarcity of standardized dietary intervention trials, and the multifactorial IBD nature make hard the development of universally effective recommendations. In this context, artificial intelligence (AI) and machine learning are emerging as transformative tools. By integrating clinical, nutritional, and microbiome datasets, AI-driven approaches can identify predictive dietary patterns, stratify patients by microbial and metabolic signatures, and guide individualized interventions. Moreover, explainable AI is beginning to reveal hidden interactions between diet, microbial communities, and disease outcomes, paving the way for precision nutrition in IBD.

Overall, nutrition represents both a challenge and an opportunity in IBD management. The integration of dietary modulation, microbiome-targeted therapies, and AI-based analytics holds great promise for transforming care, enabling earlier prevention, more personalized treatment, and long-term disease control.

SESSION 2
NEXT GENERATION PROBIOTICS: RESEARCH TRENDS

THE SCIENCE BEHIND *AKKERMANSIA MUCINIPHILA* AND ITS FUTURE PERSPECTIVE AS BENEFICIAL MICROBE

Clara Belzer

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Akkermansia muciniphila was in isolated in 2004 in an attempt to discover new gut microbiota members. This bacterium was the first indication that bacteria belonging to the phylum *Verrucomicrobia*, were present in the human gut, and lead to new 16S rRNA gene primer design for microbiota profiling. The discovery of *A. muciniphila* also led to many new insights of functionalities of bacteria in the gut. As such it led to the paradigm shift that microbes that associate with the mucosa can have beneficial functions for the host. The bacterium prompted many researchers to explore what the beneficial role to the host could be. *A. muciniphila* pasteurized form has been accepted by EFSA for save use as food ingredient. While intervention studies report that living *A. muciniphila* strains are also save for use in humans.

In this presentation, I will discuss the science behind *A. muciniphila* and its future perspective as beneficial microbe linking its capacity to reshape the gut microbial landscape, impact human health and, paving the way for innovations in commercial applications, genetic research, and probiotic development.

AKKERMANSIA MUCINIPHILA IN INFECTIOUS DISEASE: A NEW TARGET FOR THIS NEXT-GENERATION PROBIOTIC?

Cormac G.M. Gahan[1-3]

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The common gastrointestinal commensal *Akkermansia muciniphila* is a mucin-degrading bacterium that is greatly reduced in individuals consuming a high fat diet. We have shown that a high fat diet diminishes resistance to *Listeria monocytogenes* infection in mice in tandem with significantly altered immune parameters and alterations to microbiome community structure. However short-term administration of *A. muciniphila* in the context of a high fat diet reversed some of these physiological markers and rescued host resistance to *Listeria* infection.

Our studies reflect increasing evidence from a variety of pre-clinical studies which suggest that oral supplementation with *Akkermansia* can improve metabolic health, moderate systemic inflammation and potentially enhance resistance to infectious agents. Very recent studies have indicated the molecular mechanisms by which *A. muciniphila* may interact with the host to influence systemic immune-regulation and control of microbial pathogenesis. Recent studies have demonstrated the efficacy of this potential next-generation probiotic in animal models of *Salmonella* Typhimurium, *Listeria monocytogenes* and *Clostridioides difficile* as well as influenza virus and phlebovirus.

The potential mechanisms by which *A. muciniphila* may influence local and systemic immune responses will be discussed.

ONE SPECIES, MANY STRAINS: HOW CAPTURING INTRA-SPECIES DIVERSITY MAY SHAPE PROBIOTIC EFFICACY – THE CASE OF *LACTOBACILLUS CRISPATUS*

Remco Kort

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The vaginal microbiome plays an important role in reproductive health, where dominance by the bacterium *Lactobacillus crispatus* is associated with reduced risk of bacterial vaginosis, sexually transmitted infections, and adverse pregnancy outcomes. However, it is not clear if all *L. crispatus* strains confer equal benefits. Increasing evidence highlights substantial intra-species genomic and phenotypic diversity, suggesting that probiotic efficacy may depend not only on species identity but also on the diversity of strains within a formulation.

Comparative genomics of vaginal *L. crispatus* isolates has revealed considerable variability in genes involved in carbohydrate metabolism [1] and cell surface structures [2], including the presence of a fragmented glycosyltransferase gene more frequently found in strains from dysbiotic states. Building on these findings, we identified and characterized a 20 kb gene cluster in *L. crispatus* encoding a multi-component transmembrane glycosylation system, including a 5 kb-element containing a fragmented glycosyltransferase, a flippase, and UDP-galactopyranose mutase genes. This element likely underlies synthesis of specialized extracellular glycans such as exopolysaccharides or cell wall-associated glycans containing galactofuranosyl units. Experimental evolution demonstrated that this element is mobile: repeated passaging of *Lactobacillus crispatus* isolates *in vitro* led to excision of the element, flanked by identical insertion sequences, without any other functional mutations. Transmission electron microscopy revealed corresponding reductions in cell wall thickness, while preliminary FTIR-analyses indicate changes in structural cell wall polysaccharides.

These findings suggest that surface glycosylation in *L. crispatus* is a dynamic and strain-specific trait with important ecological and immunological consequences. Cell wall glycans modulate adhesion to the vaginal epithelium, biofilm formation, tolerance to low pH and antimicrobials, and interactions with host immune receptors. Strains carrying diverse glycosylation repertoires may therefore elicit distinct responses, ranging from immune evasion under dysbiotic pressure to enhanced immunomodulation in eubiotic conditions. For the host, this means that a genetically diverse community of *L. crispatus* strains may better match the heterogeneous immune landscapes found across women, thereby increasing the likelihood of probiotic efficacy at the population level.

Together, these data argue for a new approach in probiotic development: instead of relying on single ‘champion strains”, strain consortia capturing intra-species diversity, particularly in surface glycosylation capacity, may provide broader and more robust protection. Glycosylation remains a largely understudied feature in probiotic research, yet it may represent a key determinant of functional resilience and efficacy. The case of *L. crispatus* illustrates how embracing intra-species diversity could inform next-generation probiotics tailored to women’s health [3].

References

1. Hertzberger, R. *et al.*, 2022. International Journal of Molecular Sciences 23:5590.
2. Van der Veer, C. *et al.*, 2019. Microbiome 7:49.
3. Kort, R., 2025. Mixture of *Lactobacillus crispatus* strains. VU Patent Application.

LINKING INTESTINAL PERMEABILITY AND THE GUT MICROBIOME IN CIRRHOSIS: THE ROLE OF *PHASCOLARCTOBACTERIUM* AS A POTENTIAL NEXT GENERATION PROBIOTIC

Rosa Haller[1,2], N. Feldbacher[1,2], S. Fürst[1], J. Woltsche[1], L. Gulden[1], J. Schwarzl[1], J. Traub[3], T. Madl[4,5], H. Habisch[4], A. Horvath[1,2] and V. Stadlbauer[1,2,5]

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Liver cirrhosis is a leading cause of mortality in the Western world. It is characterized by increased intestinal permeability and alterations in gut microbiome composition. These changes suggest that the gut microbiome may serve as a therapeutic target for managing both liver dysfunction and intestinal barrier impairment. This study aimed to investigate the relationship between intestinal barrier integrity and gut microbiome composition in cirrhosis, and to explore potential mechanisms by which commensal bacteria influence gut permeability.

We assessed faecal zonulin, a biomarker of intestinal permeability, using ELISA, and analysed gut microbiome composition via 16S rDNA sequencing in a discovery cohort ($n = 78$) and a validation cohort ($n=90$) of patients with cirrhosis. In the validation cohort, faecal metabolome composition was further examined using NMR spectroscopy. Mechanistic insights were obtained using an intestinal barrier model based on T84 epithelial cells. In the discovery cohort (77% Child-Pugh A, 21% B, 3% C), a decrease in faecal zonulin levels over six months was associated with increased abundance of *Phascolarctobacterium*. This genus was independently linked to better liver function (lower bilirubin, $p=0.019$; INR, $p=0.019$; MELD score, $p=0.01$) and was predictive of mortality in the discovery and validation cohort. Metabolomic analysis revealed an inverse relationship between *Phascolarctobacterium* and faecal succinate, a potentially pro-inflammatory metabolite. *In vitro*, succinate increased intestinal permeability, whereas *Phascolarctobacterium succinatutens* strains improved barrier integrity, potentially by counteracting the effects of succinate.

In conclusion, *Phascolarctobacterium*, a succinate-metabolizing gut commensal, was associated with enhanced intestinal barrier function, improved liver health, reduced succinate levels, and increased 36-month survival. *In vitro*, succinate impaired barrier integrity, while *P. succinatutens* mitigated this effect. These findings suggest that *Phascolarctobacterium* may serve as a prognostic biomarker for cirrhosis and a promising next-generation probiotic for restoring gut barrier function and maintaining succinate homeostasis.

MINING THE GUT MICROBIOTA FOR NEXT-GENERATION PROBIOTIC CANDIDATES IN METABOLIC DISEASES AND BEYOND

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The gut microbiota has been linked to cardiometabolic disorders through its ability to modulate host physiology. Epidemiological studies consistently show reduced microbial diversity and decreased abundance of butyrate-producing bacteria in individuals with prediabetes and type 2 diabetes. Emerging evidence also suggests that specific gut microbiota configurations may act as reporters and predictors of health trajectories, including responses to treatment. However, the scarcity of longitudinal human data has limited efforts to identify features with prognostic potential.

To identify candidates linked to metabolic health, we analysed baseline gut microbiota in individuals with type 2 diabetes undergoing bariatric surgery, as well as in people with prediabetes tracked for disease progression. Specific taxa were enriched in patients who achieved five-year diabetes remission and in individuals with prediabetes who remained disease-free. Some of these taxa have been previously linked to healthy ageing and to gut microbiota configurations associated with reduced cardiovascular risk. These gut bacteria may represent promising candidates for next-generation probiotics with relevance for cardiometabolic health and healthy ageing.

TRANSLATING ADULT MICROBIOME COMPLEXICITY INTO HEALTH-FOCUSED BIOTICS INFORMATION

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The human gut microbiome evolves across the lifespan, shaped by ecophysiological transitions from the plasticity in infancy to the stability in healthy adulthood. This dynamic ecosystem exhibits remarkable complexity, influenced by host genetics, diet, and environmental factors, and operates across multiple taxonomic and functional scales. Microbiome composition and function align with a continuum of health states, from resilience to dysbiosis, underscoring its role as a key determinant of well-being. Advances in -omics technologies to measure molecular details combined with advanced bioinformatics and AI tools now enable quantification and decoding of these intricate patterns, integrating multi-omics and computational models to inform tailored nutrition and targeted interventions for improved health outcomes across the health and disease spectrum.

Poster pitch

P9
ROTHIA SPP. AS POTENTIAL NEXT-GENERATION ORAL PROBIOTICS: INVESTIGATION OF NITRATE REDUCTION AND ANTIMICROBIAL PROPERTIES

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For abstract, see section Poster abstracts, p. 72

SESSION 3
IPA EXPANDING GLOBAL LEADERSHIP IN THE BIOTICS SPACE

INTRODUCTION TO THE SESSION

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In 2025, the International Probiotics Association (IPA) sharpened its role in uniting the global biotics community, advancing regulatory dialogue, and reinforcing the scientific credibility of pre-, pro-, and postbiotics. Amid rapid innovation and evolving policy, IPA continues to serve as both a convener and a guardian of standards. We know the global probiotic supplements generated USD $9.3 billion in sales at the end of 2024, but market data for pre- and postbiotics remains limited due to the absence of clear, harmonized definitions, complicating market sizing and slowing the development of consistent standards. By addressing these challenges, IPA aims to emulate in pre- and postbiotics the successes it has achieved in probiotics.

The session will provide a high-level overview of IPA's current initiatives and outline potential future directions for the biotics field.

PROBIOTIC IN EUROPE: NAVIGATING A COMPLEX AND EVOLVING LANDSCAPE

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Created in 2015, IPA Europe is the European chapter of IPA. This presentation will outline IPA Europe's mission and engagement in European Commission initiatives that foster legal clarity and consumer trust. An overview will be provided of the current legal framework and practices in Europe for the labelling and marketing of probiotic food and food supplements, together with insights into the European market and consumer trends. Some key findings of the Socio-Economic Assessment (SEA) project will also be presented that quantifies the health and economic benefits of probiotics. By generating objective data, the project supports evidence-based regulation and informed decision-making with authorities and stakeholders.

COMMERCIAL PREBIOTIC MANUFACTURING: IMPLICATIONS FOR HEALTH, PRODUCT EFFICACY, AND REGULATORY APPROVALS

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Commercial scale manufacturing of prebiotics involves various processing operations tailored to the targeted product and raw material. The prevailing methods involve (i) extracting prebiotics from plant materials; (ii) enzymatic conversion via synthesis or hydrolysis; and (iii) precision fermentation. The crude extracts produced via these methods must be subsequently purified to remove byproducts and other impurities, and the purified extracts are typically concentrated and/or dried to produce finished products for commercial and consumer use. The diverse manufacturing methods used have implications on product composition, product purity, and product efficacy. Subtle differences may be observed in the degree of polymerization (impacting efficacy and dose), dictated by choice of processing conditions and raw materials.

This presentation describes some of the important and unique processes used to manufacture prebiotics, highlighting their impacts on product quality and efficacy, and impacts on regulatory approvals for products. Case studies with examples of manufacturing of select common prebiotics will be provided. This presentation highlights the unique features of different prebiotics, and how processing methods must be tailored to desired product attributes.

EATING THE ELEPHANT: IPA'S FIRST BITE IN SHAPING A CREDIBLE FUTURE FOR DTC STOOL MICROBIOME TESTING

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The direct-to-consumer microbiome testing market is the elephant in the room – enormous with inconsistent science, consumer claims, and technological standards. IPA's first steps in this space are to systematically map the global landscape of available kits for consumers to uncover gaps, inconsistencies, and existing (or lack thereof) standards.

This talk will divulge some initial takeaways from that landscape exercise and outline what concrete actions are necessary to lay the foundation for credible, evidence-based consumer testing that benefits both the industry, the scientific community, and the public.

NOVEL BACTERIA-DERIVED COMPOUNDS TO TACKLE NEURODEGENERATION: LESSONS FORM THE NEMATODE *CAENORHABDITIS ELEGANS*

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The nematode *Caenorhabditis elegans* has emerged as a powerful model in biomedical research, providing an ethically aligned and genetically tractable platform for drug discovery, toxicology, and mechanistic studies. Its short lifespan, body transparency, conserved signalling pathways, and compatibility with automated phenotyping technologies make it an invaluable system for preclinical screening in line with the 3Rs principle (Replacement, Reduction, Refinement).

As a proof of concept, we applied the SydLab One microfluidic analyser (*Nagi Bioscience*) to evaluate two bacterial-derived fermentation extracts (G1 and G2) in Alzheimer's disease (AD) nematode models. Wild-type (N2) and transgenic strains expressing human Tau (EVL1654) or Aβ1-42 (GRU102) pan-neuronally were subjected to phenotypic analysis and lifespan assays. Compounds G1 and G2 conferred striking early-adulthood benefits in the Aβ strain, significantly improving motility and reducing biological age. These effects were not observed in wild-type animals and were less pronounced in tau-expressing nematodes, with no measurable impact on overall lifespan.

Our findings suggest that G1 and G2 specifically mitigate Aβ-driven mitochondrial dysfunction rather than general ageing processes. Ongoing analyses of mitochondrial homeostasis aim to further dissect their molecular mechanisms of action. Taken together, our study underscores the utility of *C. elegans* in accelerating the identification of safe, mechanism-based interventions for neurodegeneration and demonstrates how microfluidic high-throughput platforms can bridge basic discovery and translational applications.

SESSION 4
BENEFICIAL MICROBES: PREVENTION AND TREATMENT OF MULTIPLE DISEASES

ANTIDIABETIC AGENTS AS GUT MICROBIOME MODULATORS FOR DISEASE SUPPRESSION

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Gut microbiota serves as a critical interface between diet, host metabolism, and immune regulation. While antidiabetic agents are classically regarded as modulators of host glucose homeostasis, whether and how they secondarily reshape microbial carbohydrate metabolism – and thereby influence immune responses – remains unclear. Here, we demonstrate that a widely used antidiabetic drug redirects the utilization of dietary carbohydrates within the gut microbiome, thereby attenuating mast cell-driven allergic responses in a microbiota-dependent manner. The protective effect required the presence of dietary polysaccharides. Complementary human data indicate that treatment with such agents is associated with a lower incidence of anaphylaxis, underscoring translational relevance.

Together, these findings reveal a microbiota-mediated pathway linking dietary carbohydrate metabolism to systemic immune regulation and identify antidiabetic agents as actionable modulators of the diet-microbiota-immune axis for disease suppression.

HUMAN MILK OLIGOSACCHARIDE METABOLISM BY *CLOSTRIDIUM* SPECIES SUPPRESSES INFLAMMATION AND PATHOGEN GROWTH

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Infant gut microbiome development is strongly impacted by breastmilk and human milk oligosaccharides (HMOs), with short- and long-term health implications. HMO metabolism in bifidobacteria is well-characterised, but the full range of other HMO-utilising species remains unknown. This study examined HMO-utilising bacteria that colonise preterm infants (born <32 weeks' gestation), their role in microbiome modulation, and their effects on intestinal barrier function. We found infant-derived *Clostridium perfringens*, and three other *Clostridium* species, metabolised HMOs and produced short chain fatty acids, tryptophan catabolites, and other metabolites known to improve health. We then showed that *C. perfringens* inhibited pathobiont growth and supressed inflammation in preterm-derived intestinal organoids.

These findings suggest a previously unrecognised role for *C. perfringens* strains that lack the toxin perfringolysin O in promoting healthy gut development during infancy. This demonstrates the potential of *pfoA*$^{-}$ *C. perfringens* as live or postbiotic biotherapies to suppress gut inflammation and improve intestinal barrier integrity.

PROBIOTIC INTERVENTION IN AGE-RELATED MUSCLE DECLINE: A CLINICAL PERSPECTIVE

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Older adults have long been underrepresented in clinical research, primarily to reduce data variability. However, better inclusion allows for the collection of essential data, a deeper understanding of their specific needs, and improvements in care practices. In this context, Lallemand Health Solutions has implemented a research program focused on modulating the microbiota through probiotics, with a perspective of healthy aging.

Probiotics have demonstrated beneficial effects on gut microbiota. Emerging data from preclinical and clinical studies suggest that this modulation represents a promising approach to prevent or mitigate age-related muscle loss and mobility decline. Our latest clinical study, which involved a targeted intervention on the gut-muscle axis using specific probiotic strains, showed that the tested formula could improve mobility and muscle strength, potentially delaying or preventing the onset of sarcopenia. These findings highlight the potential of probiotics to support functional autonomy and improve the quality of life of older adults throughout the aging process.

PRECISION MICROBIOME THERAPEUTICS FOR INTRACTABLE DISORDERS

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The immune system plays a central role in defending the body against pathogens while maintaining tolerance to non-harmful antigens. This delicate balance is profoundly shaped by gene–environment interactions, with the gut microbiota emerging as a key modulator. Disruptions in this commensal ecosystem are closely linked to immune and neural dysregulation, contributing to a range of disorders, including autoimmune diseases, allergies, mood disorders, autism spectrum disorder (ASD), and metabolic syndromes.

To address these challenges, we have developed Avatiome™, a platform that models the human immune system-microbiome interface in preclinical settings, enhanced by AI integration. It incorporates cutting-edge tools such as immunophenotyping, single-cell transcriptomics, and computational analysis to enable the development of precision microbiome therapeutics for intractable conditions, including neurodevelopmental disorders, systemic inflammation, and cancers. We also established IM.BERT™, a deep learning model trained on over 25,000 clinical and metagenomic datasets, enables high-resolution profiling of microbial composition, metabolic output, and host interactions. By integrating these technologies, we identified microbial strains with distinct therapeutic properties: IMB001 (immunostimulatory), IMB002 (immunoregulatory), and Lp IMB015 (neuro-immunomodulatory). We further characterized their effector molecules and mechanisms of action.

This talk will highlight the scientific rationale behind microbiome therapeutics, detailing host-microbe interactions, immune modulation, and translational potential in preclinical models.

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INTEGRATED MULTI-OMICS OF THE GUT MICROBIOME IN PARKINSON'S DISEASE REVEALS A FUNCTIONAL DISRUPTION OF BACTERIAL MOTILITY AND CROSS-FEEDING.

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Parkinson's disease (PD) is associated with gut microbiome shifts. These shifts are mainly described at the taxonomic level, but the functional and ecological consequences remain unclear. To obtain insight into the functional disruptions of the gut microbiome in PD, we used an integrated multi-omics approach, comparing gut microbiomes of individuals with PD, prodromal PD, and healthy controls.

Meta-metabolomics represented the most discriminatory and robust omic layer and was therefore selected to guide the multi-omic analyses. We identified 11 metabolites that were differentially abundant between the groups, amongst which β-glutamate was increased in PD and prodromal PD, and correlated with the transcriptional activities of Methanobrevibacter smithii and Clostridium spp. We identified decreases in transcripts, but not in gene abundances, related to glutamate metabolism, bile acid biosynthesis, chemotaxis and flagellar assembly in PD, particularly in keystone genera such as Roseburia, Agathobacter and Blautia. To gather more knowledge on the ecological modifications associated with PD, we constructed a gene co-expression network between HC and PD. This approach revealed a depletion of hub genes in PD, amongst which bacterial microcompartments, flagellar assembly and polysaccharide transport genes, which were consistently downregulated in PD. We identified a potential dysregulation of cross-feeding between distinct functional taxa along with modification of the diversity of gene expression in the PD gut microbiome.

Rather than looking at taxonomic changes in the gut microbiome. we aimed at disentangling the functional alterations that might impact PD development. Our study highlights the apparent disruption of microbial gene expression in PD, in particular for genes associated with mobility and polysaccharide use. Here, we showcase the importance of investigating the gut microbiome's functional dimensions to better resolve microbiome-host interactions in health and disease.

DATA FROM WITHIN: NAVIGATING THE SMALL INTESTINE WITH HINTSIGHT

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The small intestine (SI) remains one of the least accessible yet most critical regions of the human gut. It is the site of nutrient absorption, immune crosstalk, and bile acid metabolism, and disturbances in this environment are increasingly linked to metabolic, inflammatory, and neoplastic diseases. Yet, due to the lack of reliable sampling tools, the SI has long remained a 'black box' in microbiome science. To address this gap, we introduce HintSight, a non-invasive ingestible capsule developed by Pelican Health and evaluated within the DIGEST clinical trial. More than a sampling tool, HintSight is a platform technology designed to unlock the SI for direct exploration. The device enables triplicate fluid collection in healthy volunteers and can be equipped with a quenching formulation to stabilize fragile metabolites and microbial communities until laboratory analysis. A next-generation version further extends functionality by enabling region-specific sampling along the SI, guided by local pH values, offering unprecedented resolution of the compartment's spatial organization.

Applied in 15 individuals, HintSight enabled paired analyses that revealed the SI to be a metabolically active and microbially distinct ecosystem. Compared to faeces, the SI displayed lower species richness but enrichment in Streptococcus, Bifidobacterium, and Enterococcus. Metabolomics highlighted elevated amino acids, peptides, and conjugated bile acids in the SI, while faeces were dominated by secondary, microbe-derived bile acids. Culturomics expanded taxonomic resolution, identifying 90 cultivable species, including five potentially novel taxa.

These findings showcase the transformative potential of HintSight. By making the SI accessible through a minimally invasive, field-deployable approach, the device redefines how we can investigate gut physiology. Beyond providing new insights into host-microbe interactions, HintSight lays the foundation for a paradigm shift in microbiome research – enabling disease stratification, therapeutic development, and precision health interventions that fully account for the small intestine's central role.

Poster pitch

P14

HOW TO STUDY THE EFFECTS OF DIETARY LIPIDS ON THE SMALL INTESTINAL MICROBIOME? THE HUMAN HEALTHY FAT, HAPPY MICROBIOME (TAPIR) PROOF-OF-CONCEPT STUDY

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For abstract, see section Poster abstracts, p. 77

SESSION 5
BENEFICIAL MICROBES: THE FOCUS ON PRE- AND POSTBIOTICS

TARGETED MICROBIOME MANIPULATION: FROM METABOLIC MODELS TO PRECISION PREBIOTICS

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The microbiome’s importance is critical to host function and has been associated with several diseases. However, it is challenging to specifically promote the growth of species in away that does not support other, potentially unwanted, species. On the other hand, the microbial genomes of interest are usually available and can be used to create computational models of the respective species' metabolism. These models are used to simulate, compare, and contrast the microbial growth dynamics. Most importantly, it is possible to simulate the effect of supplementing compounds on those dynamics.

In a recent proof-of-concept study [1], the objective was to utilise genome-scale metabolic models to predict precision prebiotics. To this end, the nematode *Caenorhabditis elegans* and its related bacteria, the immune-protective *Pseudomonaslurida* MYb11 and the persistent coloniser *Ochrobactrum vermis* MYb71, were used. Specifically, we simulated the supplementation of dietary compounds to identify those that specifically support the growth of the abundance of the host-beneficial MYb11. Following the prediction of candidates, we validated the *in vitro* effect of L-serine, L-threonine, D-mannitol, and γ-aminobutyric acid. L-serine also showed a significant effect *in vivo*, paving the way for targeted therapeutics that modulate microbial abundances.

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STRUCTURE MATTERS: HOW DIFFERENT FIBRES LEAD TO DIFFERENTIAL MODULATION OF THE GUT MICROBIOTA

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Over the past several decades it has become clear that a healthy gut microbiota is crucial for host health. Dietary fibres and prebiotics have been shown to beneficially affect the gut microbiota, and through this host health. However, there are many structurally different fibres, which all influence the microbiota composition and activity in a different manner, depending on e.g., the monosaccharide composition, degree of polymerization and size in general, branching and potential decorations (such as acetylation, etc.), glycosidic linkages between sugars, solubility, fermentability.

Several years ago, we started to look at structure function relationship of fibres. Although the research is far from complete, some interesting concepts arise in either simple high-throughput models or complex, dynamic *in vitro* models. The data is fed into sophisticated AI models, to predict microbiota responses in humans to the eating of structurally diverse fibres. Challenges remain, such as interindividual microbiota composition (but much less so for activity), relative abundance versus absolute quantification (we use ddPCR for quantification), poor prediction of substrate specificity of glycosyl hydrolases (or CAZymes in general), to name a few. Structure matters, but several other factors are also important! This presentation will discuss some of these challenges and perhaps ways to solve these.

POSTIMMUNOBIOTICS: PROMISING ALTERNATIVES TO COMBAT VIRAL INFECTIOUS DISEASES IN LIVESTOCK

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Emerging and re-emerging viral diseases pose a severe threat to the productivity, economic viability, and sustainability of global livestock industries. Outbreaks produced by viruses like swine coronaviruses, classical swine fever and African swine fever in pigs has demonstrated that viral epidemics in farm animals can lead to catastrophic losses, the disruption of supply chains, and undermine food security. The traditional countermeasures including vaccines and antiviral drugs, often entail lengthy development timelines, may lose effectiveness against rapidly mutating pathogens, and can contribute to antimicrobial resistance when used prophylactically. Consequently, there is an urgent need for innovative, broad-spectrum strategies that bolster innate antiviral defences in livestock species.

Immunobiotics, microorganisms capable of beneficially modulating host immune responses, and their inanimate or functional derivatives called postimmunobiotics, have emerged as promising alternatives to enhance viral resilience in farm animals. When administered orally, immunobiotics or postimmunobiotics engage pattern recognition receptors (PRRs) expressed in epithelial cells and immune cells of the intestinal mucosa, triggering the production of type I interferons (IFN-α/β) and proinflammatory and regulatory cytokines. These signals potentiate the innate immune mechanism involved in the protection against pathogens as well as the control of detrimental inflammation, thereby strengthening both mucosal and systemic antiviral defences. In addition, it was demonstrated that some immunobiotics and postimmunobiotics are capable of stimulation the gut-lung axis and therefore improve protection not only against enteric viruses but also to viruses that infect the respiratory tract. Interestingly, postimmunobiotics can be as efficient as live microorganisms to modulate the immune system, making them a valuable tool since in addition to their biological functions they have practical advantages of simplifying storage, formulation, and regulatory compliance for use in feeds.

In this presentation, I will introduce the research of our group dedicated to the selection and characterization of immunobiotics or postimmunobiotics that have beneficial effects on the host, especially through the improvement of mucosal and systemic antiviral immunity. I will describe the construction of evaluation systems for the study of antiviral immunobiotics/ postimmunobiotics by using porcine cell lines including an originally established porcine small intestinal epithelial cell line that was successfully used for the evaluation of antiviral innate immunity. By focusing on swine as a model for livestock health, our study highlights the potential of immunobiotic-based interventions to mitigate viral threats in agriculture. Adoption of these strategies promises to reduce reliance on pharmaceuticals, curb the spread of emerging and endemic viral infections in farm animals, and contribute to more resilient and sustainable livestock production systems.

Acknowledgements
The research programme on development of innovative technology grants (JPJ007097) from the project of the Bio-oriented Technology Research Advancement Institution (BRAIN); JSPS KAKENHI No.19H00965, 23H00354, 23K18072, 25K22412, 22F22080, and 24KF0098 from the Japan Society for the Promotion of Science (JSPS).

RESTORING BALANCE: PRO- AND POSTBIOTIC STRATEGIES TO COUNTER ANTIBIOTIC-INDUCED DYSBIOSIS IN A CANINE GUT MODEL

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Gut microbiota plays a central role in dog health, supporting nutritional and physiological functions. However, antibiotic treatments can disrupt microbial equilibrium, leading to dysbiosis. While probiotic and postbiotic strategies are increasingly investigated, their use in dogs remains poorly documented, particularly in the context of antibiotic-induced dysbiosis. In this study, we provide novel insights by evaluating, for the first time, the efficacy of two microbiota restoration strategies using a canine-specific *in vitro* gut model (Canine Mucosal Artificial Colon, CANIM-ARCOL): the probiotic live yeast *Saccharomyces boulardii* CNCM I-1079 and the postbiotic heat-inactivated HA-122. Both were administered at field-relevant doses twice daily, during and after a 5-day antibiotic treatment (metronidazole/enrofloxacin), to assess their ability to enhance microbiota resistance and/or promote recovery in both lumen- and mucus-associated communities.

Within two days post-antibiotic treatment, both interventions significantly reduced the bloom of *Enterobacteriaceae* (up to −75% relative abundance, $p<0.05$), accelerated the recovery of total bacterial load (~1.5 log10 copies/ml increase), and promoted a faster restoration of bacterial diversity (Shannon index returning to baseline by day 14 vs. day 16 in the control). In addition, *S. boulardii* significantly reduced redox potential (+200 mV with antibiotic vs. +80 mV with yeast, $p<0.0001$), while *L. helveticus* preserved short-chain fatty acid concentrations (>100 mM vs 80 mM under control conditions) and upregulated beneficial metabolic pathways (e.g., norspermidine biosynthesis). Both treatments also reduced variability in microbiota profiles and enhanced functional resilience post-antibiotic exposure. Altogether, these findings provide compelling evidence for the relevance of probiotic and postbiotic strategies in companion animals and highlight the CANIM-ARCOL model as an ethical and robust alternative to *in vivo* trials for the preclinical evaluation of microbiota-targeted interventions in canine nutrition and health.

POLYPHENOLS: A NEW GENERATION OF PREBIOTICS?

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Polyphenols are a diverse class of secondary plant metabolites found in most diets and characterised by aromatic rings bearing one or more hydroxyl groups in their chemical structure. They have been associated with a range of health benefits from cardiometabolic health to immune support. The beneficial microbiome modulation effect of polyphenols is increasingly studied either alone or in combination with fibres. Recent reviews have shown that not all polyphenols have beneficial effects, choosing the right polyphenol for the right effect at the right dose is key. The specificity of polyphenols lies in their duplibiotic role on gut microbiota giving them the ability to shape the composition of the microbiota by modulating growth of specific strains; for example, anthocyanins from aronia berry have been shown to upregulate *Anaerostripes* and downregulate *Clostidium* sp. The other mode of action of polyphenols on gut microbiota is the ability of some gut bacteria to metabolise polyphenols to bioactive metabolites, for example ellagic acid can be converted to urolithin A by gram positive bacteria.

We examine a set of studies on aronia berry and its impact on vascular function and gut microbiota in healthy males, including the different impact of polyphenol and fibre-bound polyphenol. More studies are needed, particularly human clinical studies, to use more effectively this very diverse range of compounds.

HUMAN CLINICAL RESULTS ON IMMUNITY USING A POSTBIOTIC GUMMY SUPPLEMENT – A CASE STUDY FOR THE USE OF INANIMATE MICROBES IN A FOOD APPLICATION

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A postbiotic is a preparation of inanimate microorganisms and / or their components that confers a health benefit on the host. EpiCor postbiotic, derived from yeast fermentate, has been demonstrated to support immune function in adults, reducing the incidence of cold/flu-like or allergy symptoms. This randomized placebo-controlled trial assessed the incidence and severity of cold/flu symptoms and use of cold/flu medication in children (n=256) aged 4-12 years attending school/daycare, using 500 mg EpiCor or placebo in a gummy format for 84 days during the 2022-2023 flu season in Ontario. Gummy supplements are a commonly used delivery form for supplements, especially for children, but are rarely studied in clinical trials.

Cold/flu symptoms were monitored using the Canadian Acute Respiratory Illness and Flu Scale (CARIFS) questionnaire, completed daily by subjects' caregivers, and adverse events were recorded. Total CARIFS severity scores, 'sore throat' and 'muscle aches or pains' symptom scores in the EpiCor group were significantly lower compared to Placebo during incidences of cold/flu ($P \leq 0.05$). Participants taking placebo were 1.73 times more likely to use cold/flu medication compared to those receiving EpiCor ($p=0.04$). Incidence of cold/flu symptoms was not significantly different between groups. EpiCor was found to be safe and well-tolerated. EpiCor supplementation resulted in significantly lower cold/flu symptom severity and less cold/flu medication usage than placebo demonstrating a beneficial effect on immune function in children.

LUNCH BREAK SESSION

NEXT-GENERATION PROBIOTICS: PRECISION TOOLS FOR MICROBIOME ENGINEERING

Discover how microbiome engineering is reshaping animal nutrition toward more efficient, sustainable, and antibiotic-free systems. In this lunch break session, Bialtec experts will share cutting-edge research on replacing antibiotic growth promoters with microencapsulated probiotics, modulating the intestinal resistome, and developing synthetic microbiomes as a new generation of functional feed additives. Combining biotechnology, anaerobic fermentation, and gastrointestinal simulators, this lunch break session unveils practical and scientific strategies to redesign gut health from a microbiological and sustainability-driven perspective.

Presenters:
Mauricio Agudelo Rendón, CEO and Co-Founder of Bialtec
Dr Juan Esteban Vásquez, Fermentation Coordinator at Bialtec

- **Digestive antimicrobial resistance gene profile in pre-fattening pigs fed microencapsulated probiotics versus antibiotic growth promoters**

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Antimicrobial resistance (AMR) in livestock is a mounting global concern. We evaluated how microencapsulated probiotic supplementation compares with antibiotic growth promoters (AGPs) in shaping the gut resistome of pre-fattening pigs and explored links to performance. A total of 108 piglets (Landrace × Large White × Duroc; 21 ± 1 d at start) were randomly assigned to four dietary treatments from weaning to day 70: D1, probiotic premix (Fortcell Feed® Cría); D2, probiotic premix (Fortcell Feed® Ceba); D3, antibiotics (doxycycline 80 mg/kg + zinc bacitracin 50 mg/kg); and D4, copper sulphate control. Feeding was ad libitum in two phases (days 1-21 and 22-49 post-weaning). Rectal faecal samples (n=36 evaluable; days 17, 34, 51) underwent shotgun metagenomic sequencing. Reads were mapped to curated resistance databases; abundances were normalized to copies-per-million (CPM) and summarized by antimicrobial class. Co-occurrence networks (Spearman, $p<0.05$) were built for ARG–bacterial genus pairs. Principal component analysis (PCA) examined relationships between resistome structure and zootechnical metrics (final body weight, FBW; feed conversion ratio, FCR).

Across 36 samples, 14 ARG classes were detected. Aminoglycoside genes predominated across diets (≈42–49% of ARGs), followed by tetracyclines (≈17–29%) and macrolides (≈10–31%). The antibiotics diet (D3) exhibited the widest dispersion and higher extremes of ARG abundance; however, total ARG CPM did not differ significantly among diets versus control ($p>0.05$), with a trend toward higher values in D3 ($p=0.078$). ARG abundance increased with advancing post-weaning stage; stage 1 was significantly lower than stages 2-3 ($p<0.05$), with differences most evident in probiotic groups (D1, D2). Resistome burden correlated negatively with FBW ($r\approx-0.575$) and positively with FCR ($r\approx0.579$), indicating that higher ARG load aligned with poorer efficiency. Network and PCA analyses suggested distinct ecological associations and performance-linked resistome configurations across diets.

In pre-fattening pigs, the resistome is dominated by aminoglycoside, tetracycline, and macrolide genes. While microencapsulated probiotics maintained ARG levels comparable to control, AGP use tended to elevate the resistome burden without reaching statistical significance. ARG load increased with age and associated with less favourable performance metrics, supporting probiotic-based, antibiotic-sparing strategies in swine production.

- **Customized microbiomes and their potential as a new generation of functional feed additives**

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Microbiome engineering can benefit from a shift from single-strain probiotics to community-level interventions that deliver complex, durable functions. An *in vitro* approach to cultivate gastrointestinal (GI) microbiomes from target animal species in anaerobic bioreactors can allow to train these communities under defined selection pressures (such as substrates and retention time) to express desired capabilities – e.g., enhanced lactose hydrolysis or improved fibre degradation. The central manufacturing challenge is not strict taxonomic reproducibility but functional consistency. Process controls can be outlined whereby function-based release criteria (e.g., enzyme activity and short-chain fatty acid profiles) are paired with compositional 'guardrails' tracked by sequencing.

Quality-by-design elements include continuous or fed-batch CSTR operation, GI-mimetic setpoints, online monitoring (pH, ORP, gas evolution), and periodic 16S/shotgun sequencing with resistome and pathogen surveillance. It is important to address downstream hurdles – biomass concentration, washing, cryo- or spray-drying, and microencapsulation - to ensure shelf stability and gastric survival, along with dosing strategies for transient vs. colonizing applications. On the regulatory path, animal-use microbial consortia will likely be evaluated under feed/zootechnical additive or live biotherapeutic frameworks; precedents from human microbiota-based therapies and long-standing FMT policies suggest viable routes provided safety, consistency, and efficacy are demonstrated.

At Bialtec, we have a current trial of a 3 L anaerobic reactor maintaining a diverse ruminal microbiome with stable functional outputs over extended operation, currently being adapted to increase fibre degradation. The operation over 3 months has shown a stable pattern of physicochemical parameters and the first samples to check the microbiota have shown a diverse community that could be feasible to act as a functional ruminal microbiome for fibre degradation. Flocculation performed in the effluent of the reactor, together with vacuum drying and microencapsulation are being used as downstream processes to obtain a proof-of-concept product and test it in *in vitro* trials.

SESSION 6
BENEFICIAL MICROBES FOR HUMAN AND ANIMAL HEALTH

UNVEILING THE PROBIOTIC BACTERIA OF THE HONEYBEE

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Honeybees live in symbiosis with bacteria [1] that are crucial to enhancing nutrient metabolism [2,3] and immune stimulation [4,5]. However, their role as beneficial microbes is yet poorly understood, and most of the studies have focused on the bacteria that populate the gut of the honeybee, leaving out the fact that honeybees are social insects sharing many more bacteria with all the members of the beehive. In this project we propose to monitor changes in the dynamics of the microbiome of the beehive, AKA apibiome [6], a novel concept that encompass not only the bacteria present in the internal organs of the honeybee, but also those that derive from plants (nectar and pollen), soil, water and other animals living in the same ecosystem as honeybees. By knowing the bacterial communities that define the apibiome and how their presence and abundance correlate with health, we will be able to identify the key beneficial bacteria associated with protection of the whole honeybee colony.

Very recently, our group has discovered that honey carries multiple microbes representing different bacterial communities, including bee symbionts and environmental bacteria [7]. 16S rRNA sequencing of the honey DNA showed that the abundance of bee symbionts is influenced by apiculture practices. The use of antibiotics due to infections or poor hive performance is associated with a significant shift in the bacterial populations, changing from more symbionts to more environmental bacteria. Within the symbiotic population we also observed considerable alternations that correlated with antibiotic treatments. The most affected symbionts were those that are often present in the bees and hives but do not form the core gut microbiota. The beehives that did not receive any antibiotic are mainly overrepresented by *Lactobacillus kunkeei*, but this profile varied following the exposure to antibiotics. The dominance of *L. kunkeei* was shared with other bacterial species such as *Parasaccharibacteri apium* and *Arsenophonus nasoniae*. Furthermore, although the symbionts that are stably found in the gut of the honeybees (AKA core symbionts) [8-11] were detected at much lower levels, their abundance was also dependent on the use of antibiotics. Treatment with antibiotics caused more disturbance in the proportion at which these core symbionts are present, in particular that of *Gilliamella apicola*. Our results suggest that *L. kunkeei* and *G. apicola* could be acting as the probiotic bacteria of the honeybee, and to confirm this, we are currently investigating whether the fluctuations in the populations of these two symbionts affect the health of the beehive.

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PHAGE THERAPY IN POULTRY FARMING: NATURAL SOLUTIONS AGAINST ANTIBIOTIC RESISTANCE

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Antimicrobial resistance has become one of the greatest threats to public health, with projections estimating up to 10 million deaths annually by 2050. The overuse of antibiotics in both human and veterinary medicine has accelerated the rise of so-called ‘superbugs’, while the development of new antibiotics has slowed dramatically. In this context, phage therapy – a treatment discovered more than a century ago – has re-emerged as a promising solution. Bacteriophages, viruses that specifically target and kill bacteria, are the most abundant biological entities on earth. They offer several advantages over antibiotics: they act with high specificity, leaving beneficial bacteria unharmed; they are safe, eco-friendly, and naturally adaptable; and they even have the potential to reverse bacterial resistance. Despite challenges, such as the possibility of bacteria developing resistance to phages, strategies like phage cocktails, have proven effective in maintaining efficacy.

In the poultry sector, phage therapy has demonstrated promising results. It can be used both as a preventive and control strategy, showing greater effectiveness than conventional methods in reducing harmful bacteria. Studies confirm its safety and potential to improve animal health, while new commercial products are beginning to provide practical tools to limit the presence of major pathogens in poultry production. These findings show that phage therapy is a practical and innovative alternative to antibiotics in poultry production. It offers a sustainable and health-conscious approach that supports both animal health and public safety within the One Health framework. Future research is focused on optimizing its use against resistant bacteria, while regulatory pathways will be key to making this tool widely available. Phage therapy has the potential to become an effective, natural, and environmentally friendly solution for managing antibiotic-resistant infections in poultry.

SYSTEMATIC PROFILING OF THE COMPOSITION AND FUNCTION OF THE CHICKEN GUT MICROBIOME UNDER DISTINCT MICROBIAL BASED SOLUTIONS

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The overuse of antibiotic growth promoters (AGP) in the livestock industry had led to the emergence of antimicrobial resistance mechanisms (AMR), leading to global bans to eliminate the threat of AMR to global health. In search for alternatives, probiotics and other microbial-based interventions have been explored to mitigate the effects of AGP removal, including rising enteric infections caused by *Clostridium perfringens* or other foodborne pathogens that threaten food safety and security. Previous studies examining the effects of microbial-based interventions have primarily relied on 16S ribosomal DNA (rDNA) surveys focused on a single gastrointestinal (GI) site, diet and/or timepoint. This narrow approach has provided a partial understanding on the contribution of the microbiome to gut health, limiting insights to community composition, leaving community function and the eukaryotic community unobserved.

In this study, we performed a systematic investigation to evaluate how probiotic supplementation affects the chicken gut microbiome in terms of both composition and function, focusing on three microbial interventions: a complex, competitive exclusion, consortia – Aviguard, and two single-strain probiotics, the yeast *Saccharomyces cerevisiae boulardii*, and the experimental bacterium *Bacillus pumilus*. To assess their efficacy as alternatives to AGPs, we compared the effects of these microbial interventions on community composition and function against a combination treatment of bacitracin (AGP) and monensin (ionophore), commonly used therapeutics that mitigate against bacterial and parasitic diseases. Birds were raised under two different diets and 16S rDNA surveys were applied to three GI sites sampled at three key timepoints of the poultry life cycle. To gain a comprehensive understanding of microbiome composition and function, we further examined compositional changes in the eukaryotic community using 18S rDNA surveys and assessed functional shifts in the caecal microbiota through whole microbiome RNASeq (metatranscriptomics). Our findings revealed that Aviguard accelerated the early maturation of the caecal microbiota and also modulated community function, potentially enhancing the production of short-chain fatty acids. We further highlighted the potential of *S. cerevisiae boulardii* to reduce *Eimeria*, an apicomplexan parasite responsible for coccidiosis in chickens. Additionally, the interplay between diet, bird age, and microbial treatment shaped treatment outcomes and potentially prevented consistent replication of treatment effects on community composition or function across diets and developmental stages.

Overall, microbial-based products offer a promising alternative to AGP treatment, addressing global AGP bans and rising antibiotic resistance mechanisms.

MICROENCAPSULATED PROBIOTICS FOR PIGLETS: PRECISION MICROBIOME ENGINEERING AND REAL-WORLD RESILIENCE

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This presentation examines how microencapsulated probiotic consortia can precisely engineer the piglet gut microbiome to withstand production stress and improve nutrient utilization – now with a specific focus on lactose digestibility during weaning. Across controlled trials and field studies, we show that a heat- and pressure-resistant encapsulation matrix preserves probiotic viability through pelleting/storage and enables targeted release in the gut, providing robust functional delivery under commercial conditions. Drawing on recent research and field trials, the work highlights several core advances:

Microencapsulation technology. Microencapsulation protects probiotic strains – most notably *Lactobacillus* and *Saccharomyces* – from thermal, mechanical, and chemical stress during feed processing, storage, and gastrointestinal transit. This process ensures high viability and effective delivery to the gut, as demonstrated by survival rates exceeding 70% under various conditions, including temperature fluctuations, relative humidity, mechanical compression, and high salinity.

Zootechnical performance. Multiple trials in piglets show that diets supplemented with microencapsulated probiotics achieve growth rates, feed conversion ratios (FCR), and average daily gains comparable to or better than those achieved with traditional antibiotic growth promoters and trace elements (e.g., copper sulphate). Notably, probiotic-fed piglets often exhibit lower feed intake for similar weight gain, indicating improved nutrient utilization and economic efficiency.

Microbiome modulation and health. Probiotic supplementation leads to significant modulation of the gut microbiota, characterized by increased diversity (higher Shannon and Simpson indices), enrichment of beneficial genera (*Lactobacillus*, *Succinivibrio*), and suppression of pathogens such as *Escherichia coli*. Probiotic diets enriched lactose-use pathways *in vivo*, showed strong Leloir and tagatose-6P activity; and favoured downstream glycolytic flux with elevated galactose-6-phosphate isomerase, indicating complementary strategies for lactose hydrolysis and galactose catabolism. These changes are associated with enhanced gut resilience, reduced disease risk, and improved overall health.

THE RISE OF PROBIOTIC YEASTS

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Yeasts and bacteria share the fundamental characteristic of being single-celled microorganisms, yet most probiotic microorganisms on the market and in development today are bacteria, such as lactobacilli or bifidobacteria. Yeasts, however, have unique characteristics that render them a promising source of candidate probiotics. Yeasts contribute to the development of immune homeostasis, produce mediators of the gut-brain axis, and influence the composition and functions of the host microbiome. They also have cell wall components with potent health promoting functions, favourable technological properties, and unlike bacteria, are resistant to antibiotics.

Saccharomyces cerevisiae, commonly known as baker's or brewer's yeast, is a versatile microorganism used for centuries in food and beverage production. Beyond its traditional uses and its applications in biotechnology, it can bring health benefits. We screened and selected the strain *S. cerevisiae* CNCM I-3865 for a candite for IBS alleviation and proved its efficacy in 4 human clinical studies. We also investigated its potential for women's health with encouraging results on the vaginal microbiota. To discover new yeast probiotic strains, we have an ongoing a screening programme which has to date revealed tens of candidate strains from outside the *Saccharomyces* group. Yeasts are also highly amenable to genetic modifications which offers a potential way to fast track the development of new strains with specific health beneficial properties.

UNLOCKING THE MICROBIOME SIGNATURE OF ENDOMETRIOSIS: FROM DYSBIOSIS AND METALLOMICS TO TARGETED INTERVENTIONS

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Endometriosis affects millions of women worldwide, yet current approaches remain largely symptomatic rather than mechanistic or etiologic. While microbiome science has identified dysbiotic patterns in endometriosis, the field has struggled to translate findings into clinical applications due to inconsistent taxonomic reproducibility and lack of mechanistic understanding. Traditional microbiome signatures, defined merely as lists of differentially abundant taxa, fail to provide the framework necessary for therapeutic intervention. This presentation introduces a novel approach to microbiome signatures that integrates metallomics, microbial ecology, and nutritional immunity to create actionable therapeutic frameworks for endometriosis management. The goal is to demonstrate how mechanistic understanding of microbiome-metal interactions can guide targeted interventions beyond conventional taxonomic approaches.

We developed an integrated analytical framework combining microbiome profiling with metallomics analysis, focusing on the interplay between trace metals (zinc, iron, nickel, lead, cadmium) and microbial virulence factors. The approach examines how nutritional immunity factors (calprotectin, lactoferrin, transferrin, hepcidin) interact with pathogenic taxa expressing metal-dependent enzymes including zinc-dependent metalloproteases, nickel-iron hydrogenases, and glyoxalase systems. The endometriosis signature reveals elevated pathogenic taxa (*Escherichia coli*, *Bacteroides fragilis*, *Streptococcus agalactiae*, *Fusobacterium nucleatum*, *Candida albicans*) alongside decreased microbes (*Lachnospiraceae*, *Ruminococcus*). These organisms share critical characteristics: nickel-dependent glyoxalase systems enabling neutrophil evasion, iron acquisition mechanisms, oestrogen deconjugation activity, and facultative anaerobic metabolism creating pro-inflammatory microenvironments that perpetuate the chronic condition. The metallomics signature shows elevated zinc, iron, lead, cadmium, and nickel, directly supporting pathogenic enzyme function while triggering host nutritional immunity responses.

This mechanistic framework enables precise therapeutic targeting through coordinated interventions: low-nickel diets to disrupt glyoxalase function, beneficial microbes (*E. coli* Nissle 1917, *Saccharomyces boulardii*) for competitive exclusion, and novel approaches, such as hyperbaric oxygen therapy to counter anaerobic metabolism. The approach demonstrates that microbiome signatures should provide mechanistic frameworks for intervention rather than simple taxonomic lists. This methodology offers immediate clinical applicability and challenges the assumption that microbiome medicine requires decades of additional research before therapeutic implementation. This work bridges the gap between microbiome science and clinical medicine by providing replicable framework that tolerates regional heterogeneity while maintaining mechanistic coherence, potentially accelerating therapeutic development across multiple microbiome-associated conditions.

Poster pitch

P25

FLOW CYTOMETRY: A CRUCIAL TOOL FOR ASSESSING AND VISUALIZING YEAST-BACTERIA ADHESION

Marion Schiavone

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For abstract, see section Poster abstracts, p. 88

SESSION 7
FERMENTED FOODS AND HEALTH

NEUROMODULATORY POTENTIAL OF FERMENTED FOODS

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There is a growing appreciation of the health benefits of fermented foods including their potential ability to modulate the microbiota-gut-brain axis. Such foods contain diverse microbial communities and metabolites that can influence host phenotypes although the mechanisms are poorly understood especially in terms of modulating the central nervous system.

Here, we functionally annotated fermented food microbial metagenomes to identify genes potentially involved in the production of health-modulating and neuromodulatory metabolites. We identified health-associated functional diversity to be driven by food substrate categories and abiotic factors such as fermentation strategy and geographical origin, which are critical variables that influence fermented food production. We also reveal that fermented food substrates including brine, cereal, root, tuber and seed along with their food-associated microbial species to be rich in pathways pertaining to the production of neuromodulatory metabolites. Overall, we unravel key factors that shape the functional potential of fermented foods that will be an important resource for the development of precision fermented foods and beverages to enhance brain health.

DISTINCT MODULATORY EFFECTS OF HIGH-FIBRE AND FERMENTED-FOOD DIETS ON GUT MICROBIOTA, IMMUNE FUNCTION, TRANSIT TIME, AND SLEEP QUALITY IN A CITIZEN SCIENCE RANDOMIZED CONTROLLED TRIAL

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A randomized, placebo-controlled citizen science-based dietary intervention was conducted over eight weeks among 147 healthy adults to evaluate the effects of 8-week high dietary fibre (HDF) and high fermented food (HFF) diets on gut microbiota, immune function, gut transit time, well-being, and sleep quality. The HDF group significantly increased fibre intake (Δ10.3 g/1000 kcal/day) following recipes high in dietary fibre in addition of dried chicory root, while the HFF group increased fermented food consumption (+6.3 portions/day), including a fermented beverage. At the 21-week follow-up, modest improvements in fibre and fermented intake were maintained. Microbial diversity increased within the HFF and control groups, especially in HFF participants over 50 ($p = 0.04$), whereas diversity decreased in the HDF group relative to the control group. The HDF intervention enhanced butyrogenic potential by increasing *Anaerostipes*, *Faecalibacterium*, and *Bifidobacterium*, and significantly reduced gastrointestinal transit time ($p=0.01$).

A novel health benefit associated with the intake of high fibre was improved sleep quality (Athens Insomnia Scale; $p=0.03$). The HFF intervention significantly increased blood immune markers including CD8A and CD6 (T-cell activation), IL-18R1 and CD5 (immune modulation), and SIRT2, a longevity-associated deacetylase ($Q<0.05$), and induced a modest shift in the gut microbiota of participants over 50 years toward a composition characteristic of younger participants. These findings highlight distinct biological pathways through which dietary fibres and fermented foods modulate host physiology. This is the first randomized controlled nutritional intervention using a citizen science approach that demonstrates the feasibility and scientific value of engaging participants in healthier food choices.

Acknowledgements
This work was supported by the Dutch Digestive Health Fund (MDL Fonds), Amersfoort, the Netherlands under Grant WOO 22-03. The project received financial support from Keep Food Simple, and the companies Ani Biome and WholeFiber Holding BV.

A LONG HISTORY OF DAIRY: GUT MICROBIOTA AND TRADITIONAL DAIRY CONSUMPTION IN MONGOLIA

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In Mongolia, a traditional livestock-based diet rooted in nomadic culture is still maintained today. Notably, dairy production and consumption have been practiced for more than 5,000 years, forming a core part of the Mongolian way of life. In particular, the summer season coincides with the birthing and milking period of livestock, during which the intake of dairy products increases markedly. To elucidate how such a unique and seasonally shifting diet influences the human gut microbiota, we compared dietary habits and gut microbial composition between the summer and winter seasons.

Furthermore, we focused on the paradoxical fact that although most Mongolians are lactose intolerant, they regularly consume large amounts of dairy products. To investigate the relationship between dairy intake and the gut microbiota, we collected human and dairy product samples from four distinct regions of Mongolia, considering regional differences in livestock species and dairy product consumption. We analysed the composition of both dairy products and the gut microbiota and evaluated lactose-degrading capacity. Through these analyses, this study investigated the potential role of microbes from dairy and the gut in supporting a dairy-rich diet among lactose-intolerant individuals.

BEHAVIOUR OF THE MICROBIOME FOUND IN RAW MILK GOAT CHEESE DURING TRANSIT THROUGH THE GASTROINTESTINAL TRACT AS SIMULATED IN THE TIM-1 SYSTEM

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Within the consortium 'New Sustainable Agricultural Business/Revenue Models', raw milk goat cheeses are produced within a closed-cycle farming system. The microbiome was analysed throughout the entire chain, from soil to cheese. Combining traditional cheesemaking practices with modern microbiological analysis. To investigate the behaviour of the microbiome from raw milk goat cheese during gastrointestinal (GI) transit, a dynamic, computer-controlled *in vitro* model (TIM-1) was used. The TIM-1 system is a validated tool simulating the physiologically relevant digestion processes of the human stomach and small intestine.

Goat cheese samples were tested for six hours in the TIM-1, representing the average transit time through the upper GI tract. Intake (goat cheese) and hourly fractions of the ileal effluent, material emptied from the small intestine into the large intestine, were collected for analysis. Bacterial survival was assessed by viability qPCR (PMA-qPCR), and microbiota composition of viable cells was determined using 16S rDNA sequencing. The survival rate of total bacteria after passage through the stomach and small intestine was calculated and expressed as a percentage of the initial viable cell counts at the start of the experiment. In addition, the absolute abundance of the predominant taxa found in goat cheese was calculated by multiplying the relative abundance with the number of viable cells per sample fraction. The cumulative bacterial survival (as percentage of intake) after passage through the complete TIM-1 system (stomach + small intestine) was 22.10 ± 8.8%. The decrease in survival likely reflects exposure to gastric acid, bile, and pancreatic enzymes. 16S rDNA sequencing revealed that the goat cheese microbiome consisted mainly of seven bacterial genera: *Bifidobacterium*, *Lactiplantibacillus*, *Lactobacillus*, *Lactococcus*, *Lentilactobacillus*, *Leuconostoc*, and *Levilactobacillus*. All these genera are considered beneficial (potentially probiotic) for gut health. The decrease in viability for the predominant genera was approximately 1 $\log_{10}$, except for *Lactococcus*, which showed the largest reduction (> 2 $\log_{10}$). Survival rates were comparable to those previously reported for probiotics from yoghurt matrices tested in the TIM-1 model.

Acknowledgements
This research was funded by the Versnellingsagenda Noard East Fryslân, with co-funding from the Triodos Foundation and the Iona Stichting.

SESSION 8
THE BATTLE
GENETICALLY MODIFIED PROBIOTICS – TO BAN OR NOT TO BAN?

How to look at genetically modified probiotics from different perspectives? Two speakers will take a different side of the topic followed by a panel discussion and Q&A with the audience.

THE NEXT GENERATION OF PROBIOTICS ARE GMM

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The use of genetically modified microorganisms (GMM) as probiotics holds several promising applications and potential benefits. GMM could solve global health threats such as antibiotic resistance or be prescribed as personalized therapeutic agents tailored to individual needs or specific health conditions. Additionally, GMM could overcome limitations of natural probiotics such as short shelf-life or low production of desired compounds. At Lesaffre, we consider GMM technologies as a reliable alternative to produce metabolites usually obtained from endangered plant species. Through innovative GMM-adapted fermentation technology, we produce pure salidroside, a compound naturally found in the roots of *Rhodiola rosea*, known for its multiple health benefits linked to antioxidants and anti-inflammatory properties.

However, the use of GMM as probiotics raises some regulatory challenges. New genomic technics, such as site directed nuclease, have emerged recently as alternative to random mutagenesis for rapid, precise and cost-effective tools to introduce desired traits into microorganisms. Today, regulatory context of probiotics (or food products) obtained by genome-editing technologies is an international patchwork of diverse and non-harmonized local regulations. Globally, we see a tendency to simplify regulatory processes for genome-edited microorganisms, containing genetic modifications that could also be obtained naturally or by conventional technics (e.g., USA, Brazil, UK,…). However, countries are moving in this direction at different speeds. Some maintain more conservative positions on this and/or face complex political debates which slows down the adaptation of their regulations for these techniques. Public concerns about potential environmental and health risks with growing GMM generation and use have led many countries to regulate their development and commercialization. Some concerns are due to factors such as limited public understanding of science, lack of trust in developers, inadequate regulations, and poor communication about risks and benefits. Better public engagement would help bridge the gap between the views of the public and those of the biotechnology sector. In this talk, we propose to describe current regulation regarding last evolution of microbial genome-editing technologies and why, despite various concerns, the benefits-risk balance favorize the emergence of GMM as the next generation of probiotics.

WE NEED GOOD GOVERNANCE AS A PRECONDITION TO GM PROBIOTICS: LESSONS FROM THE GM PLANTS CONTROVERSY

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Genetic modification of plants is a domain of science mired in societal controversy, from early and largely unsuccessful attempts in the 1990s to persuade European citizens of the benefits of genetically modified foods to more recent policy debates on the merits of relaxing European regulations for CRISPR-induced gene edited foods. Institutional responses to societal unease have themselves evolved, from earlier assumptions that publics were ignorant, uninformed and in need of scientific education to a recognition that scientists and policymakers need to listen to publics and enter into two-way conversations.

Nevertheless, four fallacies prevail in scientific and policymaking communities that reproduce misunderstandings of societal responses and that facilitate the development of policies that are misaligned with societal values, responses and views: (i) The assumption that plants modified by gene editing techniques are no different in kind from conventionally bred plants; (ii) The assumption that the public interest, and the motivation to regulate plants modified by gene editing techniques, can be reduced to matters of risk to humans and the environment; (iii) The assumption that since these plants carry the same risks, it is proportionate that they should be subject to similar governance and oversight; (iv) The assumption that the current technology-market dyad that has dominated innovation policy since at least the WWII should be maintained: namely that in the absence of evidence of harm (determined by objective science) technologies should be permitted onto the marketplace with consumers exercising their freedom through purchasing decisions. Using evidence from in-depth societal dialogues with Dutch and UK citizens, each of these assumptions is shown to be misplaced: (i) Citizens make a clear distinction between plant varieties developed by traditional breeding and genetic techniques. For citizens, the process (and intention) of the intervention is paramount; (ii) While risk is an important value, citizens also desire fresh, wholesome, and minimally processed foods, where gene-edited foods are seen as a move away from their nature or purpose. ‘Tinkering’ in the genetic material of plants is seen as a move towards the making of plants as instrumental objects, modified for convenience and commerce; (iii) Citizens perceive Europe as having broadly got it right with strict regulations for agricultural GM technology and argue that similar precaution should be exercised with gene editing techniques; (iv) Citizens reject the technology-market dyad because they do not want GE crops to be developed purely for commercial motives driven by the logic of the market. This is not seen as likely to support the agriculture and foods we need and desire.

Having laid out the case that shows the reasonableness of public concerns to plants modified by gene editing techniques, I instantiate the lessons from previous societal controversy to the prospect of genetically modified probiotics. Rather than advocate for a ban (or not) I lay out the conditions for socially robust governance. These include a call for reflection on the object of governance (microbes vs plants), on distinctions between GM and conventional probiotics (in societal as well as in biological terms), on the interpretative framework that may be used to understand these distinctions (instrumentalism, commercialism, naturalness), and on the need for broad societal debate and what this may look like.

SESSION 9
BRAIN HEALTH AND BENEFICIAL MICROBES: WHAT'S UP, DOC?

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Deep learning algorithms, also known as neural networks, have become essential in the analysis of high-dimensionality and high complexity datasets like the ones used in the study of microbiome data. These algorithms have proven strongly efficient to unveil interactions within microbial ecosystems, standing out in tasks like pattern recognition, feature extraction or predictive modelling. Thus, they have become essential assets to study the interactions of these environments, like the gut microbiome, with physiological processes like aging and the diseases associated with it.

Aging is the greatest risk factor for the development of chronic diseases like neurodegenerative disorders or cancer. The increase in life expectancy makes it extremely urgent to understand and develop mechanisms to promote healthy aging. In that sense, the study of microbiome has emerged as an important factor in aging. Different scientific evidence points towards microbiome alterations along this process as important events in disease onset. Our research focuses on the study of the interactions of gut microbiome with aging and how alterations along the development of this physiological process can turn into neurodegenerative diseases, such as Parkinson's disease. Moreover, we are interested in the use of nutrition as a mean to influence on these events. Different nutritional interventions have shown their efficiency to modulate human microbiome and physiology due to their versatility. However, their efficiency is highly dependent on the individual's genomic and metagenomic background.

We have used diverse neural networks (NN) architectures to develop different tools: (i) NUTRIclock, an algorithm with ~3,700 whole genome shotgun (WGS) samples from public repositories, to elucidate microbiome changes with aging. It is an aging clock based on microbial profiles (relative and marker abundance) and predicted abundances for microbial metabolites. Its purpose is to calculate the biological age of a patient, based on physiological properties, in opposition to his/her chronological age and find microbiome patterns altered in the patients that could be driving potential effects of that biological age. (ii) An algorithm to detect Parkinson's disease using ~1000 WGS samples, to serve as a predictor of the disease and as a tool to identify potential microbial targets to intervene in this disorder progression. These algorithms will help us better understand the microbiome-aging- neurodegeneration interplay, further allowing us to implement personalized nutritional interventions according to a patient microbiome profile, enhancing the development of precision nutrition field.

HIGH-THROUGHPUT SCREENING OF FERMENTED FOODS FOR HEALTH BENEFITS: A SCALABLE *IN VITRO* APPROACH

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The pre-competitive Fermented Foods Consortium was set up to investigate different types of plant and dairy-based fermented foods. While fermentation has been traditionally used to preserve foods, fermented foods consumption has been associated with gastrointestinal health benefits in a variety of ways. More recently, fermented foods have also been suggested to exert beneficial effects on brain and behaviour through modulation of microbiota composition and metabolite production. In this consortium, the effects of fermented foods on the human gut microbiome were studied in the *ex-vivo* 'MicroColon' model. This 96-well high-throughput model mimicking conditions in the gastrointestinal tract has been developed over the last decade to represent and predict human colonic fermentation processes.

One aim of the project was to measure the effects of fermented foods on gut microbial saccharolytic, proteolytic and neuroactive metabolites formation by targeted metabolomics. Fermented foods were pre-digested to simulate upper gastrointestinal digestion (INFOGEST), and after ultrafiltration mimicking small intestinal absorption, digests were applied in the MicroColon model. Faecal fermentations in gut-like medium were performed with faecal inoculum of 10 individual healthy adult donors in parallel, including controls. HPLC and optimized RP-UHPLC-MS/MS (TSQ) methods were used to measure microbial metabolites in the faecal matrix, such as SCFAs, tryptophan, serotonin, GABA, histamine, dopamine, tyramine, tryptamine, indoles, and kynurenines. In addition, microbiome analyses were performed by shotgun metagenomics sequencing, and associations between microbiome composition, function, and targeted metabolomics data were assessed. The results from this study showed that exposure to pre-digested fermented food products impacted microbiota composition, with considerable variability among the donors. In addition, the fermented products exerted differential effects on metabolite production, including increased production of butyrate, GABA, serotonin, dopamine, indole propionic acid (IPA) and kynurenic acid. Levels of some of these metabolites were associated with changes in microbiota composition and/or functions.

In conclusion, the obtained results are in line with emerging insights into the potential of fermented foods to modulate gastrointestinal health and the microbiota-gut-brain axis. In addition, we show that the MicroColon model is a suitable platform for measuring the effect of ingredients on the gut microbiota and neuroactive metabolite formation. The impact of changes in microbial metabolite levels on the host, such as gut barrier functionality, immune responses, and gut-brain communication, will be studied in complementary research models. Such learnings provide mechanistic insights related to beneficial effects on health as well as predictive value for potential human intervention studies.

INITIAL INSIGHTS INTO THE MICROBIOTA-GUT-BRAIN AXIS IN EXTREMELY PRETERM INFANTS

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The microbiome is increasingly recognized as a key determinant of health and disease. In preterm infants, microbiota development is fundamentally altered, with low diversity, dominance of pathogenic bacteria and absence of beneficial species such as *Bifidobacteria* and *Bacteroidetes*. These alterations are linked to late-onset sepsis (LOS) and necrotizing enterocolitis (NEC). More recently, the microbiome has also been associated with the gut–brain axis. For preterm infant, who combine an altered microbiome with a highly vulnerable brain at risk of encephalopathy of prematurity (EoP), this link may be of particular importance. Microbial metabolites, immune activation, and gut–brain barrier signalling could directly influence brain development and long-term neurodevelopmental outcomes.

In this presentation, I will discuss the causes of altered microbiota in preterm infants, the mechanisms by which the gut–brain axis may affect neurodevelopment and possible interventions. Strategies under investigation include optimization of feeding, careful antibiotic stewardship, probiotics, and novel microbiota-based therapies. Insights from the EMINDS and Generation P studies may reveal how early-life exposures shape both the microbiome and long-term brain health.

PREBIOTICS AS PROMOTERS OF COGNITIVE RESILIENCE: A NEW PERSPECTIVE

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Prebiotics are increasingly studied for their effects on cognition, yet most human trials have focused on healthy participants under basal conditions, often yielding limited outcomes. This work, developed within the ILSI Europe Prebiotic Task Force, proposes a shift in perspective: rather than examining prebiotics merely as enhancers of cognitive performance, they could be investigated as agents that promote cognitive resilience under cognition-taxing factors such as stress, poor sleep, sedentary behaviour, or unhealthy dietary patterns. These factors have been shown to alter the gut microbiome and impair cognitive domains, making them highly relevant contexts for intervention.

We further highlight the need to include vulnerable or subclinical populations, as well as those who chronically or periodically experience such taxing factors, to better delineate the boundary conditions for prebiotic effectiveness. By broadening study populations and experimental paradigms, research can better elucidate how prebiotics may help preserve cognitive functioning and promote cognitive resilience across the lifespan.

PRECISION SYNBIOTICS TARGETING THE GUT-BRAIN AXIS IN AUTISM SPECTRUM DISORDER AND CHRONIC FATIGUE

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Current single-biotic solutions, including pre- and probiotics, are widely available and recognized for their health benefits, primarily through modulation of the gut microbiome. While their clinical efficacy is well documented, outcomes often vary due to differences in host physiology, diet, microbiome composition, and other environmental factors. This variability underscores the need for novel gut health solutions capable of delivering more consistent benefits across individuals, irrespective of interindividual microbiome differences.

Novel precision synbiotics combining pre- and probiotics as well as additional functional ingredients, can be designed to deliver robust and consistent effects across individuals. Their composition can be tailored to specific health conditions by assessing the key pillars of a given health or disease phenotype and selecting the ingredients needed to restore balance. Such formulations may harness multiple modes of action, for example: colon-targeted vitamins or short-chain fatty acids (SCFAs) to enhance microbial communication, gut barrier function, and neuroendocrine signalling; digestive enzymes to support macronutrient breakdown; or postbiotics that co-aggregate with pathogens to reduce harmful microbial colonization. Conventional immune-supporting micronutrients such as vitamin D and zinc may also be included.

Here, we highlight three recent clinical studies evaluating probiotic combinations paired with either partially hydrolysed guar gum, a blend of human milk oligosaccharides (HMOs), or fructo-oligosaccharides (FOS) plus zinc. These interventions were tested for their effects on brain health in post-COVID patients with chronic fatigue and in children with autism spectrum disorder.

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P1

The originally established PBE cell line as an *in vitro* model for investigating immunobiotics against swine influenza virus

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A porcine bronchial epithelial (PBE) cell line was established as a novel *in vitro* model for studying swine influenza virus (SIV) infection and immunity. PBE cells supported efficient replication of both SIV H1N1 and H3N2, displaying distinct cytopathic effects, including reduced transepithelial electrical resistance and cilia loss. Viral infection induced upregulation of type I interferons (IFN-β), antiviral effectors (Mx1, OAS1) and inflammatory cytokines (IL-6, IL-8), and modulated the expressions of negative regulators of the TLR signalling. The cytopathic and immunological changes induced by SIV in PBE cells resembled *in vivo* findings in pigs. The ability of *Lactobacillus acetotolerans* FST998 (LAFF998) to modulate SIV infection and immunity in PBE cells was also evaluated. PBE cells were treated with LAFF998 (MOI=10) for 24 h and then infected with SIV H3N2 or H1N1. LAFF998 significantly reduced SIV replication as shown by the reduced nucleoprotein expression levels compared to controls ($p<0.05$). LAFF998 enhanced IFN-β, OAS1 and Mx1 ($p<0.05$) and differentially modulated IL-6 and IL-8 expressions in PBE cells ($p<0.05$). Interestingly, the presence of both α-2,3- and α-2,6-linked sialic acid receptors were confirmed in PBE cells, and it was found that LAFF998 treatment reduced α-2,3-linked sialic acid receptors surface expression ($p<0.05$), suggesting a receptor-mediated antiviral mechanism. These findings demonstrate that: (i) the PBE cell line is a valuable *in vitro* model for evaluating SIV infection and immunity; and (ii) that *Lactobacillus acetotolerans* FST998 is a promising immunobiotic candidate for combating SIV infection in pigs.

P2

Unlocking the probiotic potential of *Levilactobacillus brevis*: the pivotal role of S-layer proteins in immunomodulation and barrier reinforcement

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Although relatively rare among lactobacilli, the ability to produce surface (S)-layer proteins is of great importance for their probiotic properties. As the outermost component of the bacterial cell wall, the S-layer proteins are the first point of contact of the bacterium with its microenvironment and thus directly influence the overall probiotic action of the strain. The aim of this study was therefore to characterise the role of S-layer proteins in the probiotic functions of four *Levilactobacillus brevis* strains (MB1, MB2, MB13, and MB20) isolated for the first time from the human milk microbiota. Genome analysis using the Rapid Annotation Subsystem Technology (RAST) server revealed an abundance of cell wall-associated genes in the analysed *L. brevis* strains. Functional assays demonstrated that S-layer proteins mediate autoaggregation and competitive exclusion of selected pathogens. Furthermore, exposure of Caco-2 epithelial monolayers to *L. brevis* strains or their purified S-layer proteins enhanced barrier integrity, as evidenced by increased expression of the tight junction proteins JAM-A and occludin, with the most pronounced effect observed in strain MB2 and the S-layer proteins of MB1. Strain MB20 showed the strongest binding affinity to recombinant human ZG16, while strain MB2 and MB20-derived S-layer proteins showed the greatest potential to improve the barrier function of HT29 epithelial monolayers by upregulating ZG16. Immunomodulatory profiling revealed that the strains and their S-layer proteins modulated cytokine production differently, confirming that the immunomodulatory effect of probiotics is highly strain-specific. In particular, the S-layer proteins of the MB20 strain significantly decreased pro-inflammatory IL-1β levels, while they increased anti-inflammatory IL-10 levels, indicating a shift towards an anti-inflammatory response. In summary, these results highlight the critical role of S-layer proteins in mediating adhesion and pathogen exclusion, enhancing barrier function, and modulating immune responses in *L. brevis* strains isolated from human milk, emphasising their potential for targeted probiotic applications.

P3

Development of functional fermented foods to support human health

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Fermented foods offer numerous health benefits that are not only attributed to the microorganisms they contain, but also to their metabolic products – known as microbial metabolites. These microbial metabolites significantly depend on the selection of bacterial strains used and their metabolic capacities. To explore this metabolic capacity, we compared the genomes of 600 strains derived from the Liebefeld Culture Collection, comprising >15,000 isolates mainly originated from the dairy industry, with the metagenome of the human gut microbiome in an *in silico* study. We found that 24 strains from this collection were sufficient to cover 89% of all annotated enzymatic reactions of an average human microbiome. Therefore, we hypothesized that fermented foods could be specifically produced to functionally supplement the metabolic activity of a dysbiotic gut microbiota. Consequently, we enhanced the production of certain bioactive substances (e.g., vitamins, amino acid derivatives) in fermented milk by selecting strains based on the presence of genes encoding enzymes in a targeted metabolic pathway. The functionality of the fermented milk was subsequently tested in mouse models. In particular, we showed that the immune regulatory activity of the gut microbiota could be rescued by feeding germ-free mice with a fermented milk enriched in microbial tryptophan catabolites. Finally, we developed Scoary2, an ultra-fast microbial genome-wide association studies (mGWAS) software, to link the production of specific metabolites (e.g., carnitines) to the genetic content of the bacterial strains used for the fermentation. Our approach demonstrates that the combined analysis of bacterial genomes and food metabolomes using modern bioinformatic tools offers promising potential for the targeted development of functional fermented foods.

P4

Towards precision psychobiotics: Combining *in silico* screening and metabolomic verification to identify acetylcholine-producing bacterial strains for *ex vivo* validation

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Developments in *in silico* analyses have enhanced our understanding of the mechanisms underpinning host-microbe interactions. We have recently proposed a psychobiotic discovery pipeline incorporating this information with knowledge from functional assays across *in vitro* and *ex vivo* domains to identify the most promising and rationally selected psychobiotic candidates for gut-brain axis health [1]. Acetylcholine is classically recognised as a parasympathetic neurotransmitter and can impact various physiological and behavioural processes, including immune function, cognition, and gut health. Moreover, several bacteria have been characterised for their acetylcholine-producing capability. Acetylcholine receptors (muscarinic and nicotinic) are widely expressed throughout the gastrointestinal tract, making intestinal tissue a useful model not only to characterise the effects of acetylcholine-producing strains on intestinal physiology, but also to validate the bioactivity of bacterial derived cell-free supernatants (CFS). Here, we applied a combination of *in silico* and metabolomics, and *ex vivo* analysis to identify and characterise acetylcholine-producing strains. The genomes of the strains of interest were screened using a modified version of our in-house pipeline, adapting a Gut Brain Modules (GBM) approach to identify acetylcholine synthesis potential (MGB008) [2]. Metabolomics analysis of the CFS was conducted by MS-Omics using a Thermo Scientific Vanquish LC-Orbitrap Exploris 240 MS with electrospray ionization in both positive and negative modes. Metabolites were identified using Compound Discoverer 3.3. Strains of interest that were predicted to possess the MGB008 acetylcholine synthesis GBM, with 100% pathway coverage and/or those with the highest relative intensity of acetylcholine in the CFS were selected for further *ex vivo* characterisation. Mucosa-submucosa small intestinal preparations were mounted in Ussing chambers and the acetylcholine-containing CFS from two strains were characterised for their effects on intestinal physiology, and validation of bioactivity at cholinergic receptors. CFS from both strains increased short-circuit current (Isc) following apical addition to the Ussing chamber, however, this response was insensitive to inhibition by muscarinic and nicotinic receptor antagonism. Conversely, basolateral addition of the CFS from both strains induced a concentration-dependent increase in Isc. This basolateral effect occurred on the side of the tissue where muscarinic and nicotinic receptors are located and was significantly inhibited by cholinergic receptor antagonism. Regardless of the side of addition, neither strain influenced trans-epithelial electrical resistance. These *ex vivo* intestinal physiology data provide direct evidence of the cholinergic bioactivity of the CFS derived from two bacterial strains, identified for their acetylcholine-producing potential *in silico* and verified by metabolomic profiling. The findings support further investigation of these strains for their gut-brain axis health potential.

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P5

Strain-resolved gut microbiome profiling in hours: A sequencing-free approach for precision health

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Strain-level diversity within the human microbiome plays a critical role in modulating microbial functionality, host interaction, and health outcomes. However, resolving such variation remains a major challenge in microbiome research. Sequencing-based approaches offer high-resolution insights but require deep coverage, complex bioinformatics pipelines, and extended workflows – limiting their scalability for clinical or translational applications. We present DynaMAP, a rapid, sequencing-independent platform for microbiome profiling with strain-level resolution, based on high-density optical DNA mapping. By directly imaging fluorescently labelled, high-molecular-weight DNA extracted from stool, DynaMAP generates long, single-molecule optical maps that retain structural features unique to individual microbial strains. These maps are compared against a curated reference database of ~20,000 genomes, enabling accurate strain-level identification at up to 99.8% average nucleotide identity (ANI). We validate DynaMAP across mock communities, standardized panels, and human faecal samples, showing it produces taxonomic profiles concordant with shotgun metagenomics – yet with simplified workflows, same-day results, and cost efficiency comparable to 16S amplicon sequencing. Importantly, DynaMAP eliminates the need for genome assembly or complex strain-calling algorithms, making it ideal for scalable, routine microbiome monitoring. This technology provides a new avenue for rapid and actionable insights into the gut microbiota, with direct applications in studying beneficial microbe-host interactions, microbiome-based diagnostics, and precision health monitoring.

P6

PFOS exposure correlates with decreased alpha diversity and minor alterations in microbial composition and metabolism in the human gut microbiome

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Per- and polyfluoroalkyl substances (PFAS) are persistent organic pollutants used for a wide range of applications due to their heat resistance and water repellence. Evidence has shown that several PFAS have adverse effects on human health including ulcerative colitis and inflammatory bowel disease. There is limited knowledge of the effect of PFAS on the gut microbiome. However, perfluorooctane sulphonic acid (PFOS), a now banned type of PFAS, has been shown to have a potential impact on gut microbiome composition. In this study we investigated the effect of high-level PFOS exposure on the gut microbiome of individuals consuming PFAS-contaminated meat, from locally grazing cows, in Korsør, Denmark. Serum and faecal samples were collected from exposed individuals, with PFOS concentration measured in both. 16S rRNA sequencing and metabolomic analysis was carried out on the faecal samples only. This allowed for multi-omic analysis of the effect of PFOS exposure on gut microbiome health. Our results showed that PFOS exposure negatively correlated with alpha diversity and differences in beta dispersion were found. While we saw the relative abundance of a several genera decrease, there was a significant increase in abundance for some beneficial genera including *Agathobacter*, a known short-chain fatty acid (SCFA) producer. Although we did not observe any significant correlations between *Agathobacter* and the measured SCFAs, the concentration of several SCFAs increased with PFOS concentration. Nine out of the ten SCFA acids analysed tended towards a positive correlation with PFOS concentration. For example, formic, pentanoic and propanoic acid appeared to be higher in individuals with the highest faecal PFOS concentration. Furthermore, several secondary bile acids were trended towards association with faecal PFOS concentration. While our results show changes in composition and trends towards metabolomic differences between exposure groups, no relationships were observed between beta diversity and metabolite concentration. Collectively, our results suggest that PFOS exposure affects the composition and function of the gut microbiome. However, further studies are needed to investigate the underlying mechanisms potentially driving the observed changes.

P7

Restoring gut barrier integrity: Live and heat-treated probiotic interventions in leaky gut models using *in vitro* co-culture systems

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The intestinal epithelium is shielded by tight junctions and a protective mucus layer secreted by goblet cells. This mucus barrier is the first defence against pathogens and toxins, while also mediating interactions with the microbiota. Alterations in its amount or properties, often linked to stress or dysbiosis, compromise epithelial protection and increase inflammation risk. Probiotic strains can enhance or impair mucus integrity and may help restore balance. However, their effects depend strongly on strain and dose. This study evaluates selected probiotic strains in a Caco-2/HT29 co-culture model, focusing on barrier integrity, mucus production, and tight junction expression. Most previous evidence relies on simple Caco-2 monolayer models, where the role of mucus is often overlooked; in contrast, the use of a Caco-2/HT29 co-culture allows evaluation of this additional protective barrier, providing a more physiologically relevant approach. A co-culture model of Caco-2 and HT29 cells at a 6:1 ratio was developed and optimized. To induce barrier disruption, differentiated monolayers were treated with 5% dextran sulphate sodium (DSS) for 1 h. Following damage induction, selected probiotic strains were applied either as live or heat-treated cells and incubated for 24 h. Each strain was standardized by flow cytometric counting and tested at three concentrations (10^5, 10^6, and 10^7 cells/ml) to assess potential dose-dependent effects. At the end of incubation, barrier integrity was evaluated by measuring transepithelial electrical resistance (TEER), mucus production was assessed through Alcian Blue staining, and expression of tight junction proteins (TJs), occludin and TPJ1, was quantified using ELISA. DSS treatment impaired barrier function, shown by decreased TEER, less mucus, and lower TJ protein expression vs. control ($p<0.05$). Probiotic treatment, live and heat-treated, partly restored barrier integrity. Both forms increased mucus production, up to 100% vs. damage control ($p<0.05$), especially at 10^6 live and 10^7 heat-treated cells/ml. In conclusion, live and heat-treated probiotics improve epithelial integrity by boosting mucus and restoring tight junctions. The study highlights the key, often overlooked role of dose. Probiotic concentration strongly affects the barrier, mainly via mucus, making dose optimization essential for gut therapies. Future perspectives should include transcriptomic analyses of genes involved in mucus production and tight junction regulation, which would provide deeper mechanistic insights and open the way to more targeted probiotic-based strategies.

P8

Advanced *ex vivo* gut tissue platforms to study host-microbe interactions and satiety hormone release using the InTESTine™ and intestinal explant barrier chip

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The role of the gut microbiome when predicting drug and food ingredient effects on gut and overall health outcomes is becoming more important. Furthermore, the recent development of glucagon-like peptide 1 (GLP-1) agonists such as semaglutide that stimulate GLP-1 release in the gut revolutionized how we approach weight and diabetes management. *Ex vivo* gut tissue models InTESTine™ (wells plate) and intestinal explant barrier chip (IEBC) (microfluidic organ-on-a-chip) maintain the *in vivo* distribution and properties of intestinal cell types and can integrate gut microbiota, thereby offering high translational value. Both InTESTine™ and IEBC, housing small or large intestinal tissue segments, were used to evaluate host-microbe interactions. Studies included satiety hormone release (GLP-1 and peptide YY (PYY)) upon stimulation with probiotics and microbiome-derived components, and bacterial adhesion of *Salmonella enteritidis* after 1-hour apical stimulation. An adapted IEBC format was developed to add living gut microbiota using an aerobic-anaerobic interface, and gut barrier function, transport functionality and tissue viability were studied after 24 h tissue-microbiome co-culture. *Ex vivo* intestinal tissue explants showed a 2-fold or higher increase in GLP-1 and PYY release in response to nutrients like sweetener rebaudioside A, large and small peptides. The gut microbiota's potential to stimulate GLP-1 and PYY release was demonstrated for 2 probiotic strains and microbial metabolites, including short-chain fatty acids, indole, tryptophan, and secondary bile acids. Prebiotic ingredient 2'-FL reduced *Salmonella enteritidis* tissue adhesion by 50% and decreased its translocation through the tissue. 2'-FL also significantly improved gut tissue barrier function when combined with cell-free culture supernatants from infant microbiomes, reducing the apparent permeability (P_{app}) of paracellular translocating small molecule drug mannitol by 45% under breastfed conditions and 20% under formula-fed conditions. Tissue transport functionality (P_{app} ratio caffeine/mannitol) improved 2-fold, tissue viability remained intact. A 24-hour co-culture of living human adult microbiota with colon tissue in the IEBC using an aerobic-anaerobic interface did not affect gut barrier (FD4 <0.5%/h) or transport functionality (transcellular/paracellular transport >2). Anaerobicity was confirmed by oxygen sensors (<0.5% O_2) and significant differences in microbial beta diversity compared to aerobic tissue conditions, with a higher abundance of e.g. *bifidobacterium* and *lactobacillus* species. Exposure to *Clostridium difficile* toxins induced expression of gut barrier function genes OCLN and CLDN2 and the inflammatory cytokine TNF-α. In conclusion, the InTESTine™ and IEBC are advanced models of the human intestine that can be used to study host-microbe interactions and satiety hormone release.

P9

Rothia spp. as potential next-generation oral probiotics: investigation of nitrate reduction and antimicrobial properties

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The oral microbiome is a complex ecosystem composed of multiple niches, each with distinct biochemical, chemical, and physical properties, as reflected in the microbial populations they support. Disruptions to the microbial balance, often driven by poor hygiene, dietary habits, or medication, can lead to dysbiosis and increase the risk of diseases such as periodontitis and dental caries. Prospecting new probiotics is key to developing targeted, microbiome-friendly solutions for oral health and disease prevention. This study explores the probiotic potential of *Rothia* species, a genus of commensal oral bacteria, focusing on their capacity to promote health through antimicrobial activity and nitrate-reducing functions. Using Selective Medium (ORSM), colony qPCR with *Rothia*-specific primers, and MALDI-TOF MS, we isolated 187 strains from 43 tongue biofilm samples from healthy volunteers. Notably, many of these isolates exhibited close interactions and coaggregation networks with *Streptococcus* species, suggesting that metabolic cross-feeding between *Rothia* and *Streptococcus* may be a key ecological mechanism shaping oral microbial communities. From these isolates, 48 pure cultures, including *R. dentocariosa*, *R. mucilaginosa*, and *R. aeria*, were used for further characterization and screening. These strains were evaluated for two key health-promoting traits: pathogen inhibitory activity and nitrate reduction. Importantly, an optimized nitrate reduction assay demonstrated that all three *Rothia* species could convert nitrate to nitrite, a metabolic activity with strong potential for health benefits. Nitrite and its downstream product, nitric oxide, are known for their antimicrobial effects, anti-inflammatory properties, and cardiovascular benefits. Among the isolates, *R. aeria* showed the highest average nitrite production, followed by *R. mucilaginosa* and *R. dentocariosa*, with notable strain-specific variability. Direct inhibition of pathogens was not observed under the experimental conditions tested, likely attributable to silent biosynthetic gene clusters (BGCs) and the specific parameters of the assay, including the choice of test organisms. Nevertheless, previous studies demonstrated that *Rothia* genomes are predicted to encode BGCs compounds, i.e., non-ribosomal peptide antibiotics and iron-chelating siderophores, that may support oral microbial balance when activated. Broader screening will be conducted to assess their antimicrobial potential. The observed interplay between *Rothia* and commensal *Streptococcus* species suggests that *Rothia* may contribute to a healthy oral ecosystem by supporting *Streptococcus* proliferation. This work lays the foundation for future development of *Rothia*-based interventions aimed at improving oral hygiene, reducing inflammation, and supporting cardiovascular health. Further investigation will be conducted to explore the antimicrobial potential of the genus and to elucidate the complex ecological interactions between the species within the oral microbiome.

P10

Development of *Lactiplantibacillus plantarum* as a vaccine platform against infections caused by avian pathogenic *Escherichia coli*

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Avian pathogenic *Escherichia coli* (APEC), a subtype of extraintestinal pathogenic *E. coli* (ExPEC), is a major causative agent of avian colibacillosis, leading to substantial morbidity and mortality in poultry, incurring enormous economic losses and threatening global food security. Moreover, the zoonotic potential of APEC raises public health concerns. The growing threat of antimicrobial resistance has prompted a shift toward developing alternative intervention strategies that support gut health and productivity without contributing to antimicrobial resistance. One promising approach proposes to use *Lactiplantibacillus plantarum* (LP), a probiotic lactic acid bacterium with immunomodulatory effects and potential as a live vaccine platform due to its capacity for heterologous protein expression. Genome-wide association studies combined with reverse-vaccinology tools have enabled the identification of genes encoding potential candidate antigens with the capacity to confer broad cross-protection against APEC infection. Selected candidates were cloned into three LP isolates on plasmids under a constitutive promoter, to generate candidate APEC vaccines. Using an *in vitro* model of the chicken caeca, we demonstrated that one of the promising LP isolates was able to integrate into the resident chicken caecal microbiota and be maintained for up to 120 h. Moreover, all three isolates exhibited resilience to gastric acids and bile salts, indicating their potential as orally administered vaccine vectors. These findings highlight the potential of novel APEC vaccines and innovative vaccine platforms, such as *L. plantarum*, for the safe and effective antigen expression to produce vaccines against APEC and other poultry diseases.

P11

Mucus-associated microbiota consortia: Insights from the advanced TIM-2*muc* model

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The mucus layer lining the epithelium of the colon contains a microbiome that is distinct from the luminal microbiome; this is referred to as the mucus-associated microbiome (MAM). Recently, research on the MAM has gained momentum due to its significant role in immune and metabolic health. However, the study of the MAM is limited by conventional experimental techniques, as sampling this microbiome often requires invasive procedures, making *in vivo* research challenging. Additionally, obtaining samples from the MAM can compromise the lumen, complicating simultaneous studies of both the MAM and the lumen. To address these limitations, we present the TIM-2*muc*, an advanced version of the colonic *in vitro* model TIM-2, which includes a mucus compartment comprising colonic mucus beads Gut3Beads and electrospun sheet coated with Gut3gel. To validate whether the integrated mucus compartment behaves like the *in vivo* MAM, we examined the bacterial composition, alpha and beta diversity, and the MAM's response compared to the lumen compartment of both the TIM-2*muc* and the conventional TIM-2 model. Our findings suggest that the MAM in the TIM-2*muc* model closely resembles its *in vivo* counterpart, thereby enhancing the physiological relevance of the luminal microbiome. TIM-2*muc*, an advanced TIM-2 model, presents a novel, precise, standardized, and non-invasive method for studying the MAM and LM simultaneously.

P12

Studying chicken-*Salmonella* interactions in the intestine

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Salmonella is a major foodborne pathogen responsible for severe gastrointestinal disease and poses a significant global public health concern, with poultry being the primary source of human infection. To develop strategies that limit colonization and reduce transmission risk, it is crucial to better understand the interactions between *Salmonella*, the chicken host, and the gut microbiota. *In vitro* models offer a valuable platform to pre-screen compounds before conducting animal trials. However, such models remain scarce for chickens. Here, we established in our laboratory the chicken intestinal epithelial cell line CHIC-8E11, which expresses enterocyte markers including villin, E-cadherin, and cytokeratin. We will assess its response to different *Salmonella enteritidis* strains, focusing on bacterial adhesion, intestinal barrier function, and immune responses. This model may provide a useful tool for investigating host–pathogen interactions and evaluating interventions aimed at reducing *Salmonella* colonization in poultry.

P13

Galacto-oligosaccharides promote bifidobacteria and short-chain fatty acid production at ultra-low doses

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Prebiotics are substrates selectively metabolised by the gut microbiota to support host health. In practice, efficacious intake typically ranges between 1.5-30 g/day, which often requires less convenient delivery vehicles such as powders or sachets. This work examined whether lower, capsule-compatible amounts of galacto-oligosaccharides (GOS) can exert significant effects on the adult gut microbiome. Bimuno® GOS was assessed at doses of 0.5, 0.75, 1.83, and 3.65 g using the SIFR® technology (n=8 donors). This *ex vivo* technology preserves the diversity and metabolic functionality of complex human gut microbial communities, including oxygen-sensitive and otherwise difficult-to-culture organisms. GOS was rapidly fermented and consistently increased the abundance of *Bifidobacterium* species (*B. adolescentis, B. bifidum, B. longum*), with statistically significant effects already detectable at 0.5 g. These compositional shifts were accompanied by marked increases in short-chain fatty acid (SCFA) synthesis: acetate and butyrate were statistically significantly elevated from 0.5 g and 0.75 g, respectively. Gas production remained limited, in line with the metabolic output of bifidobacteria. Beyond these responses, the resolution of the SIFR® technology revealed stimulation of additional health-associated taxa within the *Lachnospiraceae* family, including *Anaerobutyricum hallii, Bariatricus comes*, several *Blautia* species (*B. massiliensis, Blautia*_A*, B. faecis*), *Oliverpabstia intestinalis, Mediterraneibacter faecis* and *Fusicatenibacter* spp. Furthermore, enrichment of *Phocaeicola vulgatus* was linked to statistically significant increases in propionate from 0.75 g. In conclusion, this study demonstrates that GOS can elicit robust prebiotic responses at doses compatible with capsule formats, thereby enhancing the feasibility of convenient nutritional product design. The observation that even 0.5 g of the specific GOS induced bifidogenic and metabolic shifts highlights the potential for clinically relevant microbiome modulation at substantially lower intakes than previously considered necessary.

P14

How to study the effects of dietary lipids on the small intestinal microbiome? The human HealThy fAt, haPpy mIcRobiome (TAPIR) proof-of-concept study

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Emerging evidence highlights the importance of the small intestinal microbiota in digestion and metabolism, underscoring the challenging need for human studies beyond faecal analyses. This proof-of-concept study aimed to confirm the interactions between the small intestinal microbiota and dietary lipids in healthy adults after a high-fat shake challenge test. We also evaluated the impact of a plant-based mild-ketogenic preconditioning diet on microbiome composition and function. Participants consumed an 8-day plant-based mild-ketogenic preconditioning diet, followed by a high-fat shake challenge test on day 9. During this challenge test, fasting and postprandial small intestinal aspirates were collected every 20 min via a naso-intestinal catheter, while blood samples were collected hourly. In addition, participants ingested small intestine aspiration sampling capsules before (day 0) and during (day 6) the preconditioning diet, and at the start of the challenge test (day 9). Dietary compliance, capsule retrieval, sample collection, stool pattern, and gastrointestinal complaints were monitored to evaluate study feasibility. Twenty adults with a mean age of 48 years (19-88 years) and a mean BMI of 24 kg/m^2 (19.5-30 kg/m^2) completed the preconditioning diet with a 96% compliance before entering the challenge test. Aspiration capsule retrieval rate averaged 95%, with mean sample weights per timepoint of 84-95 mg and median transit times of 33-49 h. During the challenge test, the mean success rate of catheter-based aspirate collection was 49%, with large variation across postprandial time points. No significant changes in gastrointestinal complaints or stool pattern occurred throughout the study. Preliminary analyses including lipidomics and microbiome sequencing revealed distinct profiles between aspirates and faecal samples, as well as between time points, before and after preconditioning diet and during the high-fat shake challenge. This study demonstrated high feasibility with high study protocol compliance, relatively high sampling rates, and good tolerability of the dietary intervention. Sampling in the small intestine using both capsules and catheters proved achievable, each method having specific advantages and limitations. The detailed description and evaluation of our study execution provide valuable insights for designing future studies on food-microbe interactions in the small intestine. Preliminary and upcoming analyses provide insight in specific features linked to the potential interaction between small intestinal microbiota and dietary lipids (e.g. bacterial composition, diversity, and functions such as specific biosynthesis pathways) and their potential link to individual health effects.

P15

Ligilactobacillus salivarius PS21603 improves production parameters as a feed additive in weaned piglets

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After weaning, piglets undergo numerous changes (dietary, environmental, social) that result in states of stress and inflammation. These changes cause alterations and a loss of microbial diversity that can lead to structural and functional modifications in the intestine, which increases the risk of colonization by pathogens such as *Escherichia coli*. These conditions create a scenario prone to the development of diseases such as post-weaning diarrhoea, which affects the growth and fattening of piglets. Recently, the ban on the indiscriminate use of certain antibiotics and compounds such as zinc oxide has led to growing interest in the use of alternatives. In this context, the aim of the present study was to evaluate the effect of *Ligilactobacillus salivarius* PS21603 in improving the health status and productive parameters of weaned piglets. Feed additive intervention (1×10^8 CFU/day) started during the last 14 days of gestation for 16 sows while 19 sows remained as control. Supplementation was followed during lactation (28 days) and post-weaning for 42 days by their litters, 214 animals were assigned to the treated group (1×10^8 CFU/day) and 195 to the control group. Both treatment and control groups showed comparable results in terms of live-born piglets, litter weight, and average daily gain (ADG) at weaning. However, at the end of the study statistically significant differences in ADG were observed, with the treated group showing higher values (0.30 vs. 0.26; $p<0.001$). Median body weight and feed conversion ratio of piglets from PS21603 group showed statistically significant differences (22.25 kg vs. 19.66; $p<0.001$) (1.40 vs. 1.49; $p<0.001$) respectively, at the end of the transition period. The faecal microbiota composition was analysed in terms of diversity and relative abundance. By the end of the treatment period, a pronounced reduction of *Enterococcus* and *Escherichia/Shigella* genera was observed in the PS21603 group compared to the control group. Additionally, the control group showed a greater increase of *Streptococcus* and *Olsenella* genera. Importantly, *Lactobacillus* genus was significantly more abundant in the PS21603 group at the end of the study (25.03%) than in the control group (11.32%). As a conclusion, the use of *L. salivarius* PS21603 as a feed additive is effective in improving gut health and production parameters in piglets.

P16

Effects of 1,3-β-glucan from *Euglena gracilis* on gene and metabolites expression of *Lactococcus lactis*

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The prebiotic potential of 1,3-β-glucan isolated from microalgae *Euglena gracilis* was evaluated by examining its influence on the metabolic profile and gene expression of *Lactococcus lactis* DSM20175. Exposure to this insoluble 1,3-β-glucan induced significant metabolic alteration and upregulation of key enzymatic pathways implicated in carbohydrate utilization, thereby, enhancing bacterial proliferation and metabolic activity that favor gut-relevant postbiotic synthesis. *L. lactis* was inoculated into flasks containing brain–heart infusion medium supplemented with cysteine-HCl and porcine gastric mucin, and incubated anaerobically in a chamber with silicone caps. Cultures were maintained on an orbital shaker at 37 °C in the dark. The insoluble 1,3-β-glucan indicated significantly increased growth rate. Untargeted metabolomic analysis of extracellular metabolites revealed significant upregulation of 42 bioactive compounds, including metabolites with established therapeutic properties. Key metabolites linked to the gut barrier integrity and host-microbe interaction, such as lactic acid, NAD+, ornithine, shikimic acid, citrulline, 2-hydroxy-3-methylbutyric acid, 2-oxoglutaric acid, and N8-acetylspermidine were produced by the *L. lactis*. Gene expression analysis revealed coordinated transcriptional activation of polysaccharide utilization genes with the most significant upregulation observed in purN, encoding phosphoribosylglycinamide formyltransferase, a key enzyme in purine biosynthesis and DNA replication during rapid growth. Transcriptional regulators Rgg, GadR, and MutR coordinated the carbohydrate metabolic response, whereas a leucine-rich repeat domain protein appeared to mediate β-glucan recognition. Notable increases in N-acetylputrescine and 3-methylhistidine further indicated active stress adaptation and regulation of protein turnover in *L. lactis*. Although the impact of N-acetylputrescine on the gut microbiota and host intestinal epithelium remains inadequately characterized, emerging evidence suggests a role for acetylated polyamines in the regulation of intestinal permeability and stress adaptation, thereby supporting their inclusion within the expanding concept of postbiotics for functional food and nutraceutical development. These findings demonstrate that *Euglena gracilis*-derived 1,3-β-glucan functions as an effective prebiotic that induces a polyamine-enriched, carbohydrate-active metabolic phenotype in *L. lactis*, promoting gut-beneficial postbiotic production alongside the synthesis of therapeutically relevant bioactive metabolites.

P17

A combination of 4 *Bacillus clausii* strains supports the restauration of butyrate production in patients treated with statins as shown in a SHIME® *in vitro* simulation

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Statins are prescribed to lower LDL cholesterol levels in the blood. Statins can alter the composition and metabolic activity of the gut microbiota, which can influence both their therapeutic effect and side effects. Statins can significantly alter gut microbial butyrate production depending on the statin type and individual microbiome composition. The aim of this study was to investigate the protective and restorative effect of a combination of *Bacillus clausii* probiotic strains T, SIN, O/C, N/R (Enterogermina®) on the gut microbiota of patients (n=8) treated with statins (Rosuvastatin or Atorvastatin), using different configurations of the SHIME® simulation technology. In a first step, the typical SHIME® setup was adapted to a screening SHIME® configuration allowing to simulate 16 investigational arms in parallel to assess the effect of daily repeated doses of the probiotic product on the gut microbiota activity (SCFA, lactate, ammonium) and composition (16S-targeted Illumina) over a period of 8 days, as compared to a blank control. The probiotic product induced increased fermentation activity, leading to higher acetate production in 4 donors and a butyrogenic effect in the other 4 donors. The changes in acetate and butyrate production were reflected in specific enhancement of bifidobacteria and members of the *Lachnospiraceae* family. Propionate production was reduced by the probiotic treatment for all donors except one. For this donor, propionate producers, such as *Dialister* and *Bacteroides*, increased. Specific qPCR confirmed good engraftment of the *B. clausii* strains in the colon. In the second step, the probiotic treatment was co-dosed with the statins over a period of 3 weeks making use of a Quad-SHIME® inoculated with the gut microbiota of 2 donors selected from the first step with a butyrate producer and a propionate producer. As compared to the control arms, a strong butyrogenic effect was observed for both donors, especially in the distal colon. Indeed, the probiotic treatment was correlated with increased abundances of butyrate-producing genera, such as *Anaeroglobus* and *Anaerostipes*, for one donor and *Anaerostipes*, *Fusicatenibacter* and *Subdoligranulum* for the other donor. Propionate production was negatively affected by treatment, which was associated with downregulation of *Bacteroides* and *Veillonella* in both colonic regions and *Akkermansia* in the distal colon. Current data support a possible role of this combination of 4 *B. clausiia* strains in restoring butyrate production when negatively affected in patients treated with statins. Further investigations in human are required to confirm these findings and explore potential effect on statin efficacy.

P18

Analysis of bacterial translocation according to age- and Fut2 genotype-associated gut barrier dysfunction in mice

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Bacterial translocation (BT) refers to the passage of the gut microbes across the intestinal lamina propria to the other body sites that are normally sterile. BT is often associated with impaired gut integrity, which can result in heightened susceptibility to various diseases. Mucosal surface glycosylation is an important factor in maintaining gut barrier function. For example, FUT2 is an enzyme responsible for surface fucosylation, and the loss of functional *FUT2* alleles is associated with weakened gut mucosal defence, which can affect the BT occurrence. Aging can also compromise gut integrity and may trigger BT. Bacterial genomic adaptation within the host is of interest because translocating capacity of commensal microbes can be affected by age- and genotype-dependent shifts in the host's physiology. The aim of this study is to establish a robust experimental protocol for characterizing BT in a mouse model predisposed to gut barrier impairment. Our approach combines culture-independent microbiome profiling and culture-dependent isolation from mouse tissues to ensure capturing signature of translocation of viable bacteria from the gut to the extraintestinal sites. 16S rRNA gene amplicon sequencing and bacterial load evaluation by digital droplet PCR are used to characterize tissue and faecal microbiota qualitatively and quantitatively. Isolating bacteria from mesentery, liver, and stool samples using a culture-based approach can provide solid proof of translocation. Comparing the entire genomes of the isolates from tissues and faeces can help infer potential genomic adaptations of translocating bacteria. A pilot experiment was performed using three different age groups of wild-type C57BL/6J mice. The bacterial load in the tissues was orders of magnitude lower than that in the faeces, regardless of mouse age. Facultative anaerobes, such as *Lactobacillus johnsonii* and *Staphylococcus saprophyticus,* were isolated from mesenteric lymph nodes and liver. However, the isolation workflow needs further validation, as several of the isolates are suspected to be environmental contaminants. In summary, our study focuses on establishing an integrative method for investigating BT *in vivo*. This methodology will advance our understanding of the host-microbe interactions in the context of an ageing- and host genotype-related gut barrier dysfunction. This will support the development of personalized microbiome-oriented medicine in our ageing global population.

P19

The fucose-dependent rotavirus and norovirus require fucosidase activity for optimal replication

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Rotavirus (RV) and norovirus (NoV) are enteric viruses responsible for acute gastroenteritis that require fucosylated histo-blood group antigens for infection in humans. However, the way these viruses interact with intestinal fucosylated glycans to modulate infection is not well understood. Treatment of target cells with a *Bifidobacterium bifidum* α1,2-fucosidase (AfcA), which removed α1,2-linked fucose (a constituent of the H antigen from mucosal surfaces), reduced infection by RV (human Wa strain) and NoV (human stool filtrate with NoV GII.4_2012 Sydney genotype) in differentiated Caco-2 cells and in differentiated adult 3D human intestinal enteroids, respectively. Mice with antibiotic-depleted intestinal microbiota were susceptible to infection by RV_{Wa} and human NoV GII.4_2012 Sydney. Oral treatment of these mice with AfcA-loaded liposomes increased viral replication in RV- and NoV-infected animals. Conversely, the fucosidase inhibitor 1-deoxyfuconojirimycin impaired viral replication both *in vitro* and *in vivo*, highlighting the role of fucosidase activity in fucose-dependent enteric virus infection. These experiments suggest a combined contribution of both microbiota-derived and host-associated fucosidase activity in infection by fucose-dependent viruses. Our results shed light on the fundamental mechanisms governing the interplay between the host, enteric viruses, and the microbiota (a source of fucosidases). This underscores the complexity of fucose interactions for these viruses and implicates fucosidase activity as a potential antiviral target for RV and NoV.

P20

Elucidating the molecular mechanisms that commensal bacteria utilize to antagonize tuberculosis infection

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Tuberculosis (TB), being such an insidious disease, poses a burden to both humans and animals, and there is an urgent need for alternative, additional control measures to mitigate it. The complicated process by which mycobacteria strains evade host defences could be impeded if host commensal bacteria trigger cellular responses associated with pathogen clearance. We therefore hypothesise that commensal bacteria possess unique molecular mechanisms that protect the host from TB infection. In previous co-culture studies, we identified three *Lactobacillus* species, *L. plantarum*, *L. salivarius*, and *L. casei*, as capable of inhibiting *M. bovis* independent of pH. With fluorescence-activated cell sorting (FACS) analysis using porcine blood cells, we also observed that all the *Lactobacillus* species were phagocytosed by monocytes, but only *L. plantarum* decreased the internalisation of *M. bovis*. Uptake was increased by *L. casei*, and unaffected by *L. salivarius*. Finally, preliminary ImageStream analysis on the response of macrophages to PKH2-labelled *L. plantarum*, indicated that this species triggers apoptotic body formation in macrophages. In this study, we have used macrophage models to determine how commensal bacteria interact with host immune cells to influence tuberculosis infection and explain how the cell death patterns influenced by commensal bacteria influence host response to tuberculosis infection. Using differentiated THP-1 reporter cells, we have found higher activation of both the IRF3 and NF-kB signal transmission pathways in macrophages co-challenged with *M. bovis* and *L. plantarum* EML1, compared to macrophages challenged to only one or the other. With flow cytometry analysis, we have also found decreased interactions with *M. bovis* in Max-Planck Institute (MPI) cells. Overall, our study can help expand the emerging concept of commensal bacteria as a potential biological tool to combat the establishment and progression of intracellular pathogens and will also provide insight into novel microbiome strategies dedicated to fighting TB in animals and humans.

P21

Feeding a combination of postbiotics and essential oils improves broiler chicken performance and microbiota profile

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Microbiota development plays a crucial role in health and performance of farm animals. This became even more important to raise animal without relying on antibiotic growth promoters. Gut microbiome interacts with the host through various gut-organ axis, so it is of interest to find ways to positively modulate intestinal microbes to optimize production performance. Previous research demonstrated that postbiotics and phytogenics can exert beneficial effects on gut microflora, nutrient digestion as well as improved performance in animals. Two research trials were designed to test Biostrong® Dual, a unique combination of *Saccharomyces cerevisiae* fermentation postbiotic product and a blend of essential oil compounds, on performance and microbiota of broiler chickens. In both trials, a control diet (NC) and the same basal diet with on top inclusion of 400 g/MT of Biostrong® Dual (DUAL) were fed to broilers from hatch until 35 d of age. The studies used randomized block design with pens as experimental units with 24 replicate pens with respectively 20 and 16 birds per pen. At 21 d and 35 d, a cloaca swab sample was collected from one bird per pen for microbiota analysis using a commercially available platform called Galleon where key bacteria biomarkers were analysed by a fluorescence customized microarray. The main effect of treatments on performance data was analysed using JMP 18 with block nested in study as random effect, significant difference determined at $p<0.05$. At 35 d, DUAL birds had significantly higher body weight (2,745 vs. 28,07) and feed intake (3,611 vs. 3,693), while no difference in FDR (1.34 vs. 1.34). DUAL modulated the microbiota by significantly increasing signals for *Bifidobacterium* at 21 d and reducing signal of proteolytic bacteria *Sanguibacter* and *Yersinia enterocolytica* at 35 d ($p<0.05$ after FDR correction). The signal of several other proteolytic bacteria was numerically reduced by DUAL at 35 d, including *Clostridium perfringens*, *Clostridium botulinum*, *Listeria* and *Salmonella* while signal of beneficial short-chain-fatty-acid producing *Faecalibacterium* and *Ruminococcus* increased. The increase of beneficial microbes is expected to come from the postbiotic components while the reduction of proteolytic bacteria can result from less subtract driven by improved protein digestion. In conclusion, the addition of this combination of postbiotic and essential oil in broiler diets resulted in a positive modulation of the gut microbiota and improved production performance.

P22

Harnessing *Bacillus subtilis* extracellular vesicles: From bench isolation and characterization to the demonstration of immunostimulatory potential and scalable production

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Probiotics and their postbiotic derivatives, such as extracellular vesicles (EVs), are attracting growing interest due to their potential effects on various physiological functions and subsequent health applications. This work is guided around three main objectives: (i) to confirm the ability to isolate and characterize the EVs from a probiotic *Bacillus subtilis* strain, specifically the BSCU1 (CU1 registered number CNCM I-2745) strain; (ii) to explore the immunomodulatory properties of these BSCU1 EVs in vitro; and (iii) to initiate the industrial-scale transposition of the BSCU1 EVs isolation process to develop a high added-value postbiotic solution, in a responsible approach to valorizing industrial fermentation co-products. BSCU1 EVs were produced at bench-scale by clarifying and concentrating 40 ml of *B. subtilis* CU1 broth, and then isolated using the exoEasy Maxi kit (Qiagen). EVs characterization in terms of size distribution and concentration was performed on a Nanosight NS300 (Malvern). The bioactivity of the EVs was then tested on THP-1 human monocyte cell line, HEK-TLR reporter cell lines, and a 3D EpiAirway™ air-liquid interface epithelium model (MatTek), with a focus on cytokine production by ELISA and gene expression by qPCR. Next, scale-up transposition studies were carried out with 2 l fermentation bioreactor and successive tangential flow filtration (TFF strategy assessing different size and molecular weight cut-offs). The bioactivity results confirmed that the bench-scale isolated BSCU1 EVs, around 120 nm in size and at a concentration of $1*10^{10}$ to $1*10^{11}$ EVs.ml^{-1}, activate the NF-κB pathway notably via TLR2 and TLR5 receptors, inducing the production of cytokines like TNF-α. Complementary experiments on an air-liquid interface human airway epithelium revealed the induction of antimicrobial peptide (β-defensin 2), mucin (MUC5AC), and chemokine (CCL-5) gene expression, suggesting a role of EVs in reinforcing the epithelial barrier and regulating chemotactic signals. The scale-up to a liter-scale using TFF produced EVs with comparable physical characteristics to the lab-scale. In conclusion, this work suggests the boosting effect of *B. subtilis* BSCU1 EVs on mucosal innate immunity, with potential applications in human health. The scale-up process initiated for BSCU1 EVs isolation should pave the way for further development as a new postbiotic solution derived from probiotic fermentation co-products.

P23

Gut-brain axis modulation by short-chain fructo-oligosaccharides in chronically stressed male mice

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Research using various preclinical models has established the role of gut microbiota in regulating neuroendocrine and behavioural responses to stress. This study aimed to demonstrate that a diet enriched with short-chain fructo-oligosaccharides (scFOS) – prebiotic fibres – could mitigate the harmful effects of chronic stress. Adult specific pathogen-free male C3H/HeN mice were divided into three groups: (i) a control group that was non-stressed and fed *ad lib.* with a semi-synthetic diet; (ii) a group subjected to an unpredictable chronic mild stress (UCMS) procedure for eight weeks, fed the same diet; and (iii) a UCMS group that received a diet containing 100 g/kg of scFOS (Actilight® 950P; Beghin-Meiji). Five weeks after initiation of the UCMS procedure, anxiety- and depression-like behaviours were assessed. Faeces were collected during the experiment to analyse microbiota composition via 16S rRNA gene sequencing, and at the end of the study, tissues and organs were collected for biochemical and molecular analyses. Results indicate that UCMS did not significantly affect gut microbiota composition or short-chain fatty acid (SCFA) production. Still, the scFOS-enriched diet substantially altered these parameters, leading to increased SCFA levels (except valeric acid), and enhanced production of the tryptophan metabolites indole-3-propionic acid and 3-indoxyl sulphate. In the ileal mucosa, scFOS supplementation impacted the inflammatory balance through an increase in the mRNA expression of genes encoding IL-10 and IL-22, compared to the control and non-supplemented UCMS groups, and, to a lesser extent, of genes encoding TNF-α and IL-1β, compared to the non-supplemented UCMS group. UCMS triggered depressive-like behaviours, including apathy and anhedonia, but scFOS supplementation did not alleviate these behaviours. Plasma corticosterone levels increased with UCMS, while, in the hypothalamus and hippocampus, expression of the *Nr3c2* gene (coding for corticosteroid receptor) decreased, impairing negative feedback in the hypothalamic-pituitary-adrenal (HPA) axis. Additionally, there was a trend towards lower expression of the *3β-Hsd* gene in the adrenal glands, which is involved in glucocorticoid synthesis. Notably, the scFOS-enriched diet significantly reversed the effects of UCMS on these parameters, thus positively impacting the reactivity of the HPA axis. In summary, consuming a scFOS-enriched diet during chronic stress induced changes in gut health (microbiota activity, inflammatory status) and helped prevent the dysregulation of the HPA axis. Further correlation analyses will help identify relationships between microbial shifts, gut parameters, and the HPA axis reactivity.

P24

Probiotic oral care products: Probiotic presence, culturability, label claim accuracy, and effectiveness (*in vitro*)

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Per their design, several oral hygiene products have antimicrobial properties that influence the oral microbiome. However, due to overconsumption, resistance to these antimicrobials is developing. On the contrary, as a pro-microbial approach, probiotics, hold promise for rebalancing the oral microbiome towards a symbiotic state. Despite the availability of probiotic oral products, uncertainties about the probiotic presence, culturability, and reliability of label claims persist. This study assessed the culturability, effectiveness, and accuracy of probiotics listed in commercially available probiotic toothpastes and mouth rinses. A total of 16 probiotic toothpastes and 6 probiotic mouthrinses, available in the European and American markets, were analysed for the number of culturable probiotics (CFU), and if they indeed contain what they proclaim. The total bacterial load was assessed with qPCR, while culturability was assayed through plating on blood- and MRS agar. The presence of probiotic species in the products, as well as potential contaminants, were identified through sequencing. The products with viable probiotics were further evaluated for their impact on an *in vitro* oral biofilm model. Additionally, the isolated live probiotic species from these products were tested for potential inhibitory effects against selected oral pathogens. Not all of the examined products contained actual probiotics as many did not contain the described live bacteria, instead, contained ferments or inactivated postbiotics. Only 2 toothpastes found to contain culturable bacteria. Additionally, 40% of the toothpastes and about 60% of the mouthrinses contained traces of DNA from probiotics, however, these quantities were very low. Furthermore, the tested products did not show any beneficial effect on an *in vitro* oral biofilm model. Similarly, the probiotic strains isolated from the products showed no inhibitory activity against selected oral pathogens. In conclusion, This study highlights that none of the mouthrinses contained culturable probiotics, and only 12.5% of toothpaste products showed culturable strains. However, these strains had no inhibitory effect on selected oral pathogens Additionally, many products contained little quantities of probiotics, likely making these products ineffective.

P25

Flow cytometry: A crucial tool for assessing and visualizing yeast-bacteria adhesion

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Probiotic (*Saccharomyces cerevisiae)* or postbiotic (yeast derivatives like yeast cell wall enriched fractions) can benefit livestock by binding to pathogens and preventing intestinal colonization. However, these binding properties are strain-specific and may vary depending on product composition and production process. Therefore, it is crucial to develop a tool that allows for the rapid, accurate and precise quantification of yeast-bacteria complexes as well as to visualize of population heterogeneity. Complexes representing a yeast cell with attached fluorescent *E. coli* were analysed using a flow cytometer or imaging flow cytometer. We demonstrated that flow cytometry enables the measurement of interactions at a single-cell level by detecting and counting yeast cells with bound fluorescent bacteria. Imaging flow cytometry revealed that the number of bacteria attached to yeast followed a Gaussian distribution, peaking at 14. This process was shown to be rapid and nearly irreversible, with adhesion dynamics resembling a Langmuir adsorption model. Pre-incubation of *E. coli* cells with mannose abolished the interaction, and *E. coli* mutants lacking mannose-specific type 1 fimbriae no longer adhered to yeast. These findings indicate that mannose molecules in the yeast cell wall are key players in the interaction. However, not all yeast mutants with altered cell wall mannan content showed significant differences in adhesion compared to the wild-type strain, suggesting other factors are also important. Additionally, screening yeast cells from different strains and processes highlighted that adhesion depends on both the *E. coli* strain and the processing method, with the latter significantly influencing adhesion. In conclusion, the developed method enables the study of bacteria-yeast adhesion, demonstrating that the mechanism is irreversible. The results reveal significant differences in *E. coli* adhesion based on various parameters (strain, process), validating the use of flow cytometry for further screening of probiotic yeast and yeast derivatives.

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Role of 70°C heating on immunostimulatory effects of probiotic bifidobacteria in infant formula

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Bifidobacteria are key members of the infant gut microbiota, playing a critical role in the development of the immune system. As such, they are frequently added to infant formulas as probiotics to support infant health. However, commercial powdered infant formula is typically prepared with hot water at temperatures exceeding 70 °C to eliminate potential pathogenic bacteria. While this step is crucial for hygiene, it may compromise the immunostimulatory functions of these beneficial bacteria. This study examined how heating commercially available bifidobacteria strains at 70°C for 1 min (H70) – in line with WHO recommendation – impacts their ability to stimulate the immune response. Human monocyte-derived dendritic cells were exposed to heat-treated bifidobacteria, and cytokine production was measured. The results showed that heating at 70°C led to reduced cytokine induction compared to unheated bifidobacteria, yet this decline was less severe than that observed after more intensive pasteurization at 90°C for 15 min (H90). Morphological evaluation revealed that bifidobacterial cell integrity was maintained under H70, whereas H90 caused notable protein aggregation. Due to the lower incidence of aggregates under H70, we focused on the potential involvement of heat-inducible molecular chaperones, such as heat shock proteins (HSPs). Because bacterial HSPs are known to stimulate host immune cells, we assessed whether purified HSPs from Bifidobacteria retain immunostimulatory activity after the two heat treatments. Proteomic analysis identified several HSPs, with GroEL, DnaK, and IbpA being abundantly expressed in the tested bifidobacteria. These proteins were further assessed for their ability to induce cytokine production. The immunostimulatory activity of recombinant IbpA was significantly diminished after exposure to 90°C, but similar between 70°C and untreated samples. In contrast, GroEL and DnaK retained substantial heat stability in their immuno-stimulatory functions, even at H90. Additionally, TLR (Toll-like receptor) reporter assays were conducted to clarify how these proteins activate immune pathways. Heating weakened TLR2 activation for all HSPs studied. Furthermore, IbpA showed a marked decrease in its ability to activate TLR4 following heat treatment, whereas GroEL and DnaK did not exhibit this reduction. These findings suggest that incorporating live bifidobacteria into infant formula as immunostimulatory ingredients is beneficial, even after the 70°C preparation step.

P27

Targeted probiotic intervention for proton pump inhibitor-associated dysbiosis: *In vitro* screening and clinical proof-of-concept

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Proton pump inhibitors (PPIs) are among the most influential medications affecting the gut microbiome in the general population. Long-term PPI use has been linked to an increased presence of oral bacteria, such as *Streptococcus salivarius* and *Veillonella parvula*, in the gut, which is associated with intestinal inflammation and adverse outcomes in liver cirrhosis. Beyond careful prescription practices, there is a critical need for targeted strategies to mitigate PPI-induced microbiome alterations. This study aimed to develop and evaluate an evidence-based probiotic intervention to counteract PPI-associated dysbiosis. A total of 43 probiotic strains were screened for their ability to inhibit *S. salivarius* or *V. parvula* or modulate neutrophil granulocyte responses. Seven strains were selected for inclusion in a multispecies probiotic formulation. This formulation, with and without a prebiotic component, was tested in a bioreactor-based *in vitro* model of gut microbiome oralization (MyBioScope, CBmed), alongside two controls (vehicle and starch) and prebiotics alone. Subsequently, a single-arm clinical proof-of-concept study was conducted in 61 long-term PPI users (53 analysed) over a 3-month intervention period. The selected probiotic strains – *Bifidobacterium animalis* W53, *Lactobacillus acidophilus* W55, *Lactobacillus casei* W56, *Lactobacillus plantarum* W1 and W21, *Lactobacillus rhamnosus* W71, and *Pediococcus acidilactici* W143 – demonstrated inhibitory effects on *S. salivarius* and *V. parvula* and modulated neutrophil responses. In the MyBioScope *in vitro* gut microbiome model, the probiotic formulation significantly reduced *V. parvula* abundance and showed a non-significant trend toward reducing *S. salivarius*. Neither prebiotics alone nor control substances affected oral bacterial abundance. In the clinical study, the probiotic intervention significantly improved participants' gastrointestinal quality of life and mirrored *in vitro* findings, with a significant reduction in *V. parvula* and a modest, non-significant reduction in *S. salivarius*. Adherence and safety were excellent. In conclusion, this study presents a rationally designed, evidence-based probiotic formulation targeting PPI-induced dysbiosis. The selected strains effectively reduced *V. parvula* abundance in both an *in vitro* bioreactor model and a clinical setting, with additional benefits for gastrointestinal well-being. These findings support the use of targeted probiotic strategies to mitigate microbiome-related side effects of long-term PPI therapy.

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From microbial ecology to dietary interventions: metabolic modelling of the chicken gut microbiome

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The gut microbiome plays a crucial role in host health and productivity, yet the mechanistic understanding of microbial interactions remains limited. With the global phase-out of antibiotic growth promoters in poultry production, understanding gut microbiome dynamics is essential for developing effective alternatives. We applied community-scale metabolic modelling to investigate chicken gut microbiome function and predict dietary intervention outcomes. Using metagenomic data from 33 chicken ceca, stratified by *Bacteroides* abundance into high-*Bacteroides* and no-*Bacteroides* groups, we constructed 237 metagenome-assembled genomes. Metabolic modelling of communities built from these genomes generated profiles of short-chain fatty acids (SCFA) largely consistent with experimental assays and supported the role of *B. fragilis* as a keystone taxon, central to the production of metabolites consumed by other taxa. In its absence, communities exhibited substantial functional reorganization with redistributed cross-feeding and the emergence of alternative hubs that assumed metabolic roles typically fulfilled by *B. fragilis*. Moreover, metabolic adaptations in *Bacteroides*-depleted communities shared metabolic similarities with profiles seen in dysbiotic states in humans. These findings highlight how the loss of keystone taxa can restructure trophic interactions and potentially impact host nutrient supply. Building on these insights, we developed a six-compartment model encompassing the entire gastrointestinal tract from gizzard to colon. This comprehensive framework integrates feeding/fasting cycles, transit-time-driven motility (peristalsis and reverse peristalsis), oxygen constraints, and nutrient absorption, enabling prediction of dietary intervention effects throughout the gut. The model captures spatial and temporal dynamics of microbial metabolism, revealing how upstream processes influence downstream communities. Screening 64 different compounds identified L-threonine, cellulose, and starch as consistently effective in promoting SCFA production. We then evaluated model predictions against targeted metabolomics from feeding trials where chickens were supplemented with these three compounds. Initial six-compartment simulations showed partial agreement with measured caecal metabolite shifts, while a trial-informed two-compartment model parameterized with 16S profiles from ileum and cecum improved recovery of the expected treatment responses in directions and relative magnitudes of metabolite changes. This work establishes a comprehensive multi-compartment model of the chicken gut microbiome, revealing hierarchical organization of microbial interactions and providing a predictive tool for developing targeted nutritional strategies in sustainable poultry production.

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Targeting the gut microbial choline–TMA pathway: Functional potential of novel probiotic strains in cardiometabolic health

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Over the past decades, the gut microbiota has been recognized as a key regulator of both physiological and pathological processes. Among the conditions shaped by microbial activity, cardiovascular diseases (CVD) have received particular attention, owing to the recognition that specific microbial metabolites contribute to their onset and progression. One of the most extensively studied is trimethylamine N-oxide (TMAO), generated through microbial metabolism of dietary choline, carnitine, and phosphatidylcholine, followed by hepatic oxidation. Elevated circulating levels of TMAO are consistently associated with atherosclerosis, vascular inflammation, thrombosis, and major adverse cardiovascular events. The choline TMA lyase pathway, encoded by the *cutC* and *cutD* genes, represents the core enzymatic machinery for TMA formation and has become an attractive therapeutic target. Since choline is ubiquitous in the human diet and endogenously secreted in bile, strategies aimed at reducing its microbial conversion into TMA are increasingly explored. In this context, probiotics represent a promising approach, as they can modulate microbial community structure and function, suppress detrimental metabolic pathways, and enhance protective ones. This study investigated three probiotic strains, *Bifidobacterium breve* BR03 (DSM 16604), *Lactiplantibacillus plantarum* LP14 (DSM 33401), and *Limosilactobacillus reuteri* LRE11 (DSM 33830), through an integrated genomic and functional analysis. Whole-genome sequencing was performed to identify genes linked to oxidative stress resistance, adhesion, bile salt hydrolase (bsh) activity, exopolysaccharide biosynthesis, and degradation of antinutritional factors (AFN). The presence of genes associated with TMA production (*cutC, cutD, cntA*) was specifically investigated. In parallel, *in vitro* assays were conducted to evaluate cholesterol-lowering capacity of the strains. Genomic analysis revealed that none of the tested strains carried *cutC, cutD,* or *cntA*, the canonical genes for TMA production. Instead, alternative choline uptake and metabolism pathways were identified, suggesting the potential to divert substrates away from TMA formation and indirectly attenuate TMAO accumulation. Functional assays demonstrated that all three strains were effective in reducing cholesterol levels. Among them, LP14 exhibited the most comprehensive genomic profile, including nearly complete folate biosynthesis pathways and an extensive repertoire of adhesion-related genes, supporting both persistence in the gut and contribution to nutrient bioavailability. Collectively, the results support the potential application of *B. breve* BR03, *L. plantarum* LP14, and *L. reuteri* LRE11 as probiotic-based strategies to modulate gut microbial metabolism, reduce TMAO-associated cardiovascular risk, and promote overall metabolic and cardiovascular health.

P30

Probiotic use is correlated with self-reported improvements in female health and wellbeing throughout life

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Probiotics are increasingly used to support female health, particularly during ageing where perimenopause and menopause take course. Probiotics have been shown to elicit their function across multiple health axes and positive outcomes are often observed in people with disorders of gut brain interaction (DGBI). Therefore, it is postulated that the effects of probiotics on female health could be impactful not just via urogenital interactions, but through gut-brain interactions also. In a real-world study, 4,002 participants across the USA (n=2,000) and UK (n=2,002) took part in a general health survey to assess (i) self-reported health metrics including stress, anxiety, depression, mental and physical health and gastrointestinal symptoms during probiotic supplementation; and (ii) current use of probiotics in the context of these metrics. An exploratory sub-group analysis was performed with females only (n=2,000) of which three age categories were defined; pre-menopause range ('Pre', age 18-44, n=964), perimenopause range ('Peri' age 45-54, n=346) and post-menopause range ('Post', age 55+, n=690). Within these groups, 323 (34%), 132 (38%) and 239 (36%) respondents, respectively, were identified as currently using (PbC) as opposed to previously using (PbP) probiotics. Self-reported mental health score comparisons revealed improvements in the PbC-Pre (p=0.03) and PbC-Peri (p=0.003) groups compared with their respective PbP groups. Self-reported stress (p=0.005), anxiety (p=0.002) and depression (p=0.0002) scores were also improved in the PbC-Peri group compared with respective PbP-Peri groups. There were trends for self-reported depression scores to be improved in both the PbC-Pre (p=0.06) and PbC-Post (p=0.06) groups. Respondents were sub-grouped according to their DGBI status through Rome IV assessment. In the PbC-Pre DGBI group, there was a trend for depression scores to be improved (p=0.07). In the PbC-Peri DGBI group, depression (p=0.0005), stress *(*p=0.03) and mental health scores (p=0.01) were improved and there was a trend for anxiety to be improved (p=0.06). In the PbC-Post DGBI group, physical health (p=0.01), depression (p=0.02) and stress (p=0.04) were improved. Taken together, this exploratory analysis suggests a correlation between continuous probiotic usage and self-reported improvements in gut-brain health and wellbeing in women, particularly in age groups where perimenopause is typically experienced.

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Engineering lactic acid bacteria to diminish mercury toxicity

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Exposure to mercury (Hg) through diet poses a significant health risk. Various strains of lactic acid bacteria (LAB) have the ability to bind the inorganic (Hg^{2+}) and organic (CH_3-Hg^+) forms of mercury most commonly found in food, thereby reducing their bioaccessibility in the gastrointestinal tract. LAB rarely possess mercury detoxification mechanisms. We introduced the *merA* (Hg^{2+} reductase), *merB* (CH_3-Hg^+ demethylase), and *merETP* (Hg membrane transporters) genes into *Lacticaseibacillus paracasei* in various combinations and tested its resistance to different forms of mercury. Expression of *merB* showed no effect on Hg^{2+} toxicity. However, in strains containing *merB*, CH_3-Hg^+ proved much more toxic compared to controls. The toxicity of both Hg^{2+} and CH_3-Hg^+ was drastically reduced by the presence of *merAB* or *merETP-AB*. In contrast, *merETP* genes alone increased the toxicity of Hg^{2+}. The *merETP-A* combination conferred greater resistance to Hg^{2+}, while showing no effect on CH_3-Hg^+. Culturing *L. paracasei* with 0.25 ppm of CH_3-Hg^+ showed that a strain carrying *merETP-AB* or *merAB* was able to eliminate more than 95% of mercury from the medium, whereas the presence of only *merA*, did not diminish Hg^{2+} contents. These findings align with the hypothesis that CH_3-Hg^+ has greater cellular permeability than Hg^{2+}. Thus, when organic mercury is demethylated intracellularly by MerB to yield Hg^{2+}, it results in a more toxic mercurial species compared to CH_3-Hg^+. On the other hand, the presence of MerETP transporters facilitates the entry of Hg^{2+} into bacteria, which, in the absence of subsequent transformation, also leads to increased toxicity. However, co-expression of Hg transporters (MerETP), MerB, and MerA enables the following sequence: extracellular Hg → intracellular Hg, followed by transformation CH_3-$Hg^+ \rightarrow Hg^{2+}$ (demethylation by MerB) and/or $Hg^{2+} \rightarrow Hg^0$ (reduction by MerA). This leads to the formation of a much less toxic mercury species (metallic Hg), which is volatile and can be eliminated from the medium. The results suggest that LAB strains expressing *merETP-AB* genes could be useful in detoxifying CH_3-Hg^+, the mercurial species associated to fish products, thus serving as tools to eliminate the most harmful forms of mercury in the intestine. This would result in reduced oxidative and inflammatory epithelial damage caused by this heavy metal, as well as decreased absorption and accumulation in tissues.

Acknowledgments
This work was funded by the Ministry of Science, Innovation and Universities/Spanish State Research Agency (PID2022-137342OB-I00) and co-financed by the European Regional Development Fund.

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